Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

Phase II Study of Neoadjuvant Imatinib in Glioblastoma: Evaluation of Clinical and Molecular Effects of the Treatment

Evangelia Razis,[1] Panayotis Selviaridis,[2] Stephanos Labropoulos,[1] Jeremy L. Norris,[6] Mei-Jun Zhu,[7] David D. Song,[7] Thea Kalebic,[8] Michael Torrens,[1] Anna Kalogera-Fountzila,[3] George Karkavelas,[4] Sofia Karanastasi,[5] Jonathan A. Fletcher,[7] and George Fountzilas[5]

Abstract

Purpose: Phase I-II studies indicate that imatinib is active in glioblastoma multiforme. To better understand the molecular and clinical effects of imatinib in glioblastoma multiforme, we conducted a neoadjuvant study of imatinib with pretreatment and posttreatment biopsies.

Experimental Design: Patients underwent a computerized tomography-guided biopsy of their brain tumors. If diagnosed with glioblastoma multiforme, they were immediately treated with 7 days of imatinib 400 mg orally twice daily followed by either definitive surgery or re-biopsy. Pretreatment and posttreatment tissue specimens were tested by immunohistochemistry for Ki67 and microvessel destiny, and posttreatment specimens were analyzed for the presence of intact imatinib in tissue. Furthermore, pretreatment and posttreatment pairs were analyzed by Western blotting for activation of platelet-derived growth factor receptor, epidermal growth factor receptor (EGFR), phosphoinositide 3-kinase/AKT, and mitogen-activated protein kinase signaling pathways. Pharmacokinetic studies were also done.

Results: Twenty patients were enrolled. Median survival was 6.2 months. Intact imatinib was detected in the posttreatment tissue specimens using mass spectrometry. There was no evidence of a drug effect on proliferation, as evidenced by a change in Ki67 expression. Biochemical evidence of response, as shown by decreased activation of AKT and mitogen-activated protein kinase or increased p27 level, was detected in 4 of 11 patients with evaluable, matched pre- and post-imatinib biopsies. Two patients showed high-level EGFR activation and homozygous *EGFR* mutations, whereas one patient had high-level platelet-derived growth factor receptor-B activation.

Conclusions: Intact imatinib was detected in glioblastoma multiforme tissue. However, the histologic and immunoblotting evaluations suggest that glioblastoma multiforme proliferation and survival mechanisms are not substantially reduced by imatinib therapy in most patients. (Clin Cancer Res 2009;15(19):6258–66)

Authors' Affiliations: [1]1st Department of Medical Oncology, Hygeia Hospital, Athens, Greece; [2]Department of Neurosurgery, "AHEPA" Hospital, [3]Department of Radiology, "AHEPA" Hospital, [4]Department of Pathology, and [5]1st Department of Internal Medicine, Oncology Section, "AHEPA" Hospital, Aristotle University of Thessaloniki School of Medicine, Thessaloniki, Greece; [6]Mass Spectrometry, Protein Discovery, Inc., Knoxville, Tenessee; [7]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; and [8]Novartis Oncology, East Hanover, New Jersey

Received 7/17/08; revised 6/5/09; accepted 6/5/09; published OnlineFirst 9/29/09.

Grant support: Novartis and the Hellenic Cooperative Oncology Group.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Evangelia Razis, Department of Medical Oncology, Hygeia Hospital, Er. Stavrou & Kifissias Av., 151 23 Maroussi, Athens, Greece. Phone: 302106867165; Fax: 306867175; E-mail: e.razis@hygeia.gr.

doi:10.1158/1078-0432.CCR-08-1867

Glioblastoma multiforme is the most common central nervous system tumor in adults and is highly malignant, with a median survival of 16 months from diagnosis. Usually, treatment includes surgery, postoperative radiotherapy, and chemotherapy with temozolomide and/or alkylating agents.

Several growth factors drive and sustain gliomagenesis, among them platelet-derived growth factor (PDGF) and its receptor, PDGFR. Different isoforms of PDGF are expressed in variable amounts, as is the case for PDGFR (1, 2).

In high-grade gliomas, epidermal growth factor (EGF), its receptor (EGFR), and transforming growth factor a (TGFa) are overexpressed, leading to autocrine and paracrine stimulatory loops (3).

Imatinib blocks the ATP binding site of the tyrosine kinase proteins BCR, ABL, KIT, and PDGFR, thus inhibiting their activation and the transduction of downstream signals. Imatinib

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

Translational Relevance

The understanding of the molecular and biochemical effects of imatinib in glioblastoma multiforme may contribute to a better tailoring of treatment with imatinib in this or other tumors, both by the identification of patient subgroups most likely to benefit from such treatment and by the understanding of all related pathways. Furthermore, this form of drug-efficacy testing (neoadjuvant therapy with pretreatment and posttreatment biopsies) could serve as a model for future testing of other targeted therapies in other tumors.

has clinical activity in various diseases (4–6), and KIT has been reported to be activated in glioblastoma cells and glioblastoma endothelial cells (7). In fact, the KIT downstream signaling effectors, m-TOR and AKT, have also been found to be overexpressed in glioblastoma multiforme (7). Imatinib activity has been shown in glioblastoma multiforme cell lines (8) and mouse models (9). Phase I and II trials have only yielded modest results, but this could be due to the fact that a targeted agent was used in an unselected population, i.e., in a nontargeted fashion (10, 11). A phase II study of imatinib in combination with hydroxyurea generated promising results, although the treatment outcome cannot be attributed solely to imatinib (12).

Although the biochemical effects of imatinib have been well elucidated in gastrointestinal stromal tumors and chronic myelogenous leukemia, the molecular effects of imatinib treatment on glioblastoma multiforme are poorly understood.

This study was designed to investigate the clinical and biological effects of imatinib therapy in glioblastoma multiforme and to verify the inhibition of imatinib targets and their downstream pathways.

We measured the concentration of imatinib in glioblastoma multiforme tissue using mass spectrometry–based proteomic technology (13), and then evaluated the effect of the drug on proliferation using Ki67 as a marker. Biochemical response, as manifested by changes in the differential activation status pretherapy and posttherapy of several signaling molecules, was also evaluated by Western blotting in an effort to determine which signaling pathways are dysregulated by imatinib in the tumor.

Finally, pharmacokinetic studies were done and serum levels of imatinib (and its metabolite) were correlated with the tissue levels. Those parameters were then associated with microvessel density (MVD) in pretreatment and posttreatment specimens.

Patients and Methods

The protocol was approved by the Institute and Ethics committees of both hospitals involved in the study and by the Greek National Organization of Medicines, Division of Pharmaceutical studies and research (Study Identification number: CST1571BGR03). The participants gave informed consent, and the study was conducted under International Conference on Harmonisation guidelines for Good Clinical Practice and the declaration of Helsinki. Inclusion criteria included age >18 y, measurable disease by magnetic resonance imaging, adequate end-organ function, and life expectancy of ≥3 mo.

Patients with newly diagnosed brain tumors that were radiogically consistent with glioblastoma multiforme were evaluated clinically and, if eligible, were accrued to the protocol. The tumor was biopsied under computerized tomography (CT) guidance. Once glioblastoma multiforme diagnosis was confirmed, the patient was started on imatinib 400 mg orally, twice daily for 7 d (14 doses). Corticosteroid dose had to remain stable throughout the 7 d, and the only antiepileptic agent allowed was valproic acid because it does not induce the CYP3A4 major p450 human enzymes that catalyze imatinib biotransformation.

Six to twenty-four hours after the last dose of imatinib the patients underwent definitive (cytoreductive) surgery or, if not resectable, a second CT-guided biopsy. For two patients, however, surgery was delayed. For the first patient, the delay was due to intensive care unit unavailability, whereas the second patient developed pulmonary complications that required hospitalization and mechanical support.

Each time six specimens were obtained. Two or more of them were snap-frozen, whereas the rest were fixed in paraffin.

Immunohistochemistry. Tumor samples from 20 patients were tested for proliferative activity and MVD by immunohistochemistry. The material consisted of formalin-fixed paraffin-embedded tissue blocks of pretreatment surgical biopsies as well as after-treatment surgical biopsies. However, three of the pretreatment samples and two of the posttreatment samples, five tissue samples in total, were nonevaluable due to extensive necrosis. The pretreatment MVD was not evaluated in an additional case, because after testing for Ki67 the remaining material was insufficient for further analysis.

Immunohistochemical assays for CD34 (clone QBEnd/10, Novocastra) and Ki-67 (clone MIB-1, Dako) were done.

Scoring system for interpretation of immunohistochemical markers

Ki67 (MIB-1). Ki67 immunostaining was scored as previously described (14). Briefly, 500 neoplastic cells were counted in 10 representative fields (hot points) using a high-power (×40) objective with a grid screen. All stained cells were recorded as positive irrespective of the staining intensity. The percentage of positively stained cells, termed the proliferation index, was calculated and recorded.

CD34. Intratumoral MVD was assessed as previous described, with slight modifications (15). Specifically, nonnecrotic tumor areas of highest neovascularization (hot spots) were found by scanning the tumor sections at low-power magnification (×4 or ×10 total magnification). After the identification of highest neovascularization areas, microvessel counting was carried out on three high-power fields and expressed as the mean MVD. Any single endothelial cell or cluster of endothelial cells was counted as a single microvessel. Muscle vessels were not counted. The mean value for the three fields with MVD was recorded as the MVD for each tumor and expressed as the number of vessels of three counts.

Pharmacokinetics. Blood specimens for pharmacokinetics were obtained on the final day of treatment. Specifically, trough levels were drawn 11 h after the 13th or 14th dose of imatinib. After centrifugation at 1,500 *g* plasma was stored at -20°C.

Imatinib mesylate and its major active metabolite *N*-desmethyl CGP4588 were measured at the reference laboratory of Novartis Pharma in Paris, France. The previously established technique using liquid chromatography and mass spectroscopy for imatinib and its metabolite was used (16, 17).

Quantitation of imatinib in tumor samples. Quantitation of imatinib in tissue was done using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) on individual patients' postsurgery samples. Additionally, four presurgery samples were pooled for use as blanks and to make representative calibration samples. Tissue sample homogenates were made by combining tissue and PBS in a ratio of 1:4 (mg/μL). Prior to protein precipitation, particulates are removed from samples by centrifugation (3,000 *g*, 5 min). All samples were analyzed in triplicate, including blank samples and quality control samples containing 1,000 μg/mL imatinib

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

Table 1. Biochemical response to imatinib

aa	CODE	Surgery after treatment	Radiotherapy after protocol treatment	Temozolomide	SURVIVAL (months)	STI571 (11-12 hours after imatinib administration, ng/ml)	CGP74588 (11-12 hours after imatinib administration, ng/ml)	Average tissue concentration (ng/g)
1	001/1	biopsy	Yes	YES	8.40			
2	001/2	biopsy	Yes	YES	17.30	1510	300	
3	002/1	biopsy	Yes	YES	17.80			
4	002/2	partial	Yes	NO	6.50	655	292	1426
5	003/1	biopsy	Yes	YES	11.50			
6	003/2	biopsy	No	NO	1.10	2420	415	2786
7	004/2	partial	Yes	YES	11.90	1240	335	1609
8	005/2	biopsy	Yes	YES	5.90			1916
9	006/2	partial	No	NO	2.30	1870	445	1180
10	007/2	total	Yes	NO	5.50	1380	368	1463
11	008/2	biopsy	Yes	YES	17.90			1379
12	009/2	partial	Yes	YES	7.90			2923
13	010/2	partial	Yes	YES	11.30	1730	458	
14	011/2	partial	Yes	YES	5.20	2090	543	3223
15	012/2	partial	No	NO	2.80			1190
16	013/2	partial	Yes	YES	6.20			1530
17	014/2	biopsy	No	NO	3.80	1250	421	1254
18	015/2	partial	No	NO	2.20	2630	557	1340
19	016/2	partial	No	NO	2.20			1928
20	017/2	partial	No	NO	6.50	1020	504	2023

and deuterium 8–labeled imatinib (d8-imatinib). For analysis of the drug the Thermo TSQ Quantum, Surveyor HPLC system was used and a HPLC Column: XTerra RP C18, 100 mm × 2.1 mm (Waters) was applied.

The average imatinib concentration was computed using the coefficients from the linear regression, and the errors (SD) were reported. Standard compounds, imatinib and d8-imatinib, were provided by Novartis. The methodology employed for this study was similar to that described by Titier et al. (16). The limit of detection for imatinib was <1 ng/mL of tissue homogenate, and the limit of quantitation was 10 ng/mL. It was found that these results compare favorably with imatinib assays previously reported for plasma (13, 16). For quality control purposes, a sample containing 1,000 ng/mL of both imatinib and d8-imatinib and a blank (no imatinib) sample were measured at the beginning of the experiment, end of the experiment, and between each replicate measurement of the patient sample set ($n = 4$). Blank injections (data not shown) confirmed that no detectable carryover of imatinib was observed.

Western blotting and reagents. Cell lysate preparations from frozen tumors, protein quantization, electrophoresis, and immunoblotting were carried out as described previously (18, 19). Protein expression levels in glioblastoma biopsies were compared pretreatment and posttreatment with imatinib. Chemiluminescence signals were captured and quantified using a FUJI LAS1000plus system with Science Lab 2003 ImageGauge V2.2 software (Fuji film Medical Systems). Polyclonal rabbit antibodies to phospho-KIT Y703, mitogen-activated protein kinase (MAPK) p42/44, and phospho-EGFR were from Invitrogen Laboratories. Polyclonal rabbit antibodies to phospho-AKT S473, total AKT, and phospho-MAPK p42/44 T202/Y204 were from Cell Signaling; phospho-PDGFR, total PDGFRα and β, KIT, and EGFR were from Santa Cruz; phosphoinositide 3-kinase (PI3-K) p85 was from Millipore. Mouse monoclonal anti-p27 (clone 57) was from BD Transduction Laboratories.

Results

A total of 20 patients, 14 male and six female, were assessed. Median tumor size was 51 mm in the greatest diameter. Two patients had a performance status (PS) on the Eastern Cooperative Oncology Group (ECOG) scale of 0, nine had a PS of 1, and nine patients had a PS of 2. Histology showed mixed

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

Table 1. Biochemical response to imatinib (Cont'd)

Biochemical Response	pAKT (pre-treatment biopsy)	pAKT (post-treatment biopsy)	AKT (pre-treatment biopsy)	AKT (post-treatment biopsy)	pMAPK (pre-treatment biopsy)	pMAPK (post-treatment biopsy)	MAPK (pre-treatment biopsy)	MAPK (post-treatment biopsy)
Decreased AKT activation	++	+	+++	+++	+	-	+++	+
Not Evaluated (Poor quality of pre-IM biopsy)	-	+	-	+	-	+	-	++
	+++		++		++		++	
Not Evaluated (Poor quality of pre-IM biopsy)	-	+++	-	+++	-	+++	-	+++
No biochemical response-although increased p27	+++	+++	++	+++	+++	+++	+++	+++
No biochemical response	+++	+++	+++	+++	+++	+++	+++	+++
Not Evaluated (Poor quality of pre-IM biopsy)	-	+	-	+	-	++	+	++
No biochemical response	+	-	-	-	+	-	-	-
No biochemical response	++	++	+	+	++	++	+	+
No biochemical response	+	+	++	+	+	+	++	+
Decreased MAPK activation	+++	++	++	+	++	+	+++	++
No biochemical response	-	-	-	-	-	-	+	-
	-		+		-		+	
	-		-		+		+	
No biochemical response-although increased p27	++	-	++	-	+++	-	++	-
No biochemical response	+	-	+	-	-	+	+++	+++
	++		+++		+++		+++	
No biochemical response	+	+	+	+	+	+	++	++

glioblastoma multiforme and astrocytoma in 3 patients and pure glioblastoma multiforme in 17.

Clinical (radiologic) response was assessed by magnetic resonance imaging on the 7th day of therapy. In 18 patients the tumor remained stable, whereas in 1 patient disease progressed and 1 patient was nonevaluable because he did not undergo posttreatment magnetic resonance imaging.

Median survival was 6.2 months (range, 1.1-17.9 months). Twelve patients underwent a partial or total resection after treatment and eight underwent a second biopsy only. Only 13 of the 20 patients received standard adjuvant temozolomide chemotherapy and radiotherapy. The reasons for not having standard treatment were (*a*) poor PS and early death in five patients, and (*b*) refusal/loss to follow-up in two. Three patients had alkylator-based therapy after relapse and one patient survived an exceptionally long period of 18 months.

Grade III toxicity was respiratory in two, urinary tract infection in one, fatigue/anemia in two, central nervous system in three (one seizure, one dysarthria, and one epidural hematoma), and poorly controlled glucose in one patient with diabetes mellitus.

Pharmacokinetics. The median trough level of imatinib (STI 571) was 1,510 ng/mL (range, 655-2,630 ng/mL); the median trough level of CGP 74588 was 421 ng/mL (range, 292-557 ng/mL).

Availability of good-quality tissue specimens for testing was limited by the small size of the biopsy specimens, which additionally, as expected in glioblastoma multiforme, had areas of necrosis in them. However, there were 14 good-quality, matched pretreatment and posttreatment specimen pairs for biochemical analysis, and in 15 cases there was posttreatment tissue available for imatinib tissue level measurement.

Quantitation of imatinib in tissue. The imatinib analysis in the tissue confirmed the feasibility of applying LC/MS/MS to evaluate the drug level. Results are shown in Fig. 1. It was found that the measurement of imatinib was linear with respect to concentration across a minimum of three orders of magnitude. ($r^2 = 0.9992$). The limit of detection for imatinib was <1 ng/mL

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

Table 1. Biochemical response to imatinib (Cont'd)

aa	pPDGFR (pre-treatment biopsy)	pPDGFR (post-treatment biopsy)	PDGFRA (pre-treatment biopsy)	PDGFRA (post-treatment biopsy)	PDGFRB (pre-treatment biopsy)	PDGFRB (post-treatment biopsy)	pKIT (pre-treatment biopsy)	pKIT (post-treatment biopsy)	KIT (pre-treatment biopsy)	KIT (post-treatment biopsy)
1	-	-	+	-	+	+	-	-	+	-
2	-	-	-	+	-	-	-	-	-	-
3	-		+		-		-			
4	-	-	-	+	+	+++	-	-	-	++
5	-	-	-	-	+	+	+++	+++	+	+++
6	+++	+++	-	-	++	-	-	-	-	-
7	-	-	-	++	-	-			-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	+	+	+	+++	-	-	-	-
10	-	-	-	-	+++	+	-	-	-	-
11										
12	-	-	-	-	++	+	-	-	-	-
13	-	-	-	-	-	-			-	-
14		-		-		-		-		-
15		-		-		-		-		-
16	-	-	-	-	+	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-
18		-		++		++		-		-
19										
20	-	-	-	-	-	-		-	-	-

of tissue homogenate, and the limit of quantitation was 10 ng/mL. It was found that these results were in agreement with previously reported levels of imatinib in plasma. For quality control purposes, a sample containing 1,000 ng/mL of both imatinib and d8-imatinib and a blank (no imatinib) sample were measured at the beginning of the experiment, end of the experiment, and between each replicate measurement of the patient samples ($n = 4$). The blank results (data not shown) confirmed that no detectable carryover of imatinib was observed. Additionally, replicate measurement of the standard showed that the ratio of [imatinib]/[d8-imatinib] was not substantially changed (CV = 1.94%) after measurement of >75 samples.

The amount of imatinib in each of the treated patients was determined using LC/MS/MS, and imatinib levels were found to range between 1,180 and 3,323 ng/g of tumor tissue, with a median value of 1,530 ng/g.

There was no correlation between trough plasma levels of imatinib and average tissue imatinib levels (Spearman's $r = 0.07$, $P = 0.865$).

Furthermore, no correlation was identified between CGP74588 levels and average imatinib tissue concentration (Spearman's $r = 0.05$, $P = 0.898$).

Three of the twenty patients showed low average tissue concentration relative to their CGP74588 concentration. None of these patients showed a biochemical response, whereas their MVD remained relatively low posttreatment.

These three patients survived for 2.2, 2.3, and 3.8 months.

Immunohistochemistry. Ki67 pretreatment and post treatment was assessed as a marker proliferation. No identifiable change in Ki67 expression was detected. Furthermore, CD34 was tested as a marker of tumor vascularity (MVD), in order to correlate it with tissue levels of imatinib. There was a slight identifiable upward trend in MVD posttherapy without any significant correlation to serum or tissue levels of imatinib.

No significant correlation between the pretreatment MVD and the average imatinib tissue levels was detected (Spearman's $r = -0.15$, $P = 0.628$).

Biochemical response to imatinib. Biochemical responses to imatinib were characterized by examining oncogenic AKT and MAPK signaling pathways. Of the 20 patients studied, 16 showed variable AKT and MAPK phosphorylation, ranging from marginally detectable to high. Of 14 patients with matched pre- and post-imatinib treatment biopsies, 3 were not evaluable because of unequal cell protein levels in pretreatment and posttreatment samples (Table 1). Among the 11 analyzable patients, expression of the p27 antiproliferation checkpoint protein increased after imatinib treatment in two patients (Fig. 2). Immunoblotting quantitations showed that AKT phosphorylation (assessed as ratio of phosphorylated to total AKT) decreased 7.6-fold in the post-imatinib biopsy in patient 001/1, whereas MAPK per molecule phosphorylation decreased 1.1-fold in patient 009/2 (Fig. 2). Patient 003/2 had high-level PDGFRB activation, which was not inhibited after imatinib treatment (Fig. 3), nor was AKT or MAPK inhibited by imatinib treatment in this individual. We could not show a *PDGFRB* genomic mutation in exons 11, 13, and 17 (data not shown) in this tumor. The tumors from patients 003/1 and 007/2 showed high-level EGFR activation, and denaturing high-performance liquid chromatography mutational analyses and sequencing studies revealed homozygous *EGFR* exon 3 (R108K) and exon 15 (G598V) missense mutations in these tumors, respectively.

Discussion

The role of imatinib in glioblastoma multiforme has been studied in combination with radiotherapy and hydroxyurea, and as a single agent, in several trials designed to examine clinical efficacy in the general glioblastoma multiforme population

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

Table 1. Biochemical response to imatinib (Cont'd)

pEGFR (pre-treatment biopsy)	pEGFR (post-treatment biopsy)	EGFR (pre-treatment biopsy)	EGFR (post-treatment biopsy)	p27 (pre-treatment biopsy)	p27 (post-treatment biopsy)	PI3K (pre-treatment biopsy)	PI3K (post-treatment biopsy)	ki67 change (post-pre)	MVD change (post-pre)
-	-	+	-	+	-	+++	++		
-	+++	-	+	-	++	-	+		
++		+++		++		++		-4	-11
-	+	-	+	-	+++	+	+++	-4	322
+	+	+++	+++	+	+++	++	++	-6	-159
+	+	++	+	++	++	+++	+++	-2	
-	+	-	-	-	+++	+	+	10	211
-	+	-	-	-	-	+	+		
-	-	+	-	++	++	+++	+++	13	48
-	+++	+	+++	+	++	+++	+	19	447
								-6	-89
-	-	-	-	++	++	++	++	-34	7
-	-	-	-	-	-	++	++		
	+		+		+++		+	12	259
	-		-		+		+	-6	-134
+	-	+	-	+	+++	+++	+	-13	405
-	-	-	-	+	+	+++	+	23	-32
	-		+		+		+++		
								-6	129
-	+	+	+	+	+	+++	+++	-2	150

without selecting for a particular target (11, 12). The lack of clinical efficacy observed in those studies could have been explained in several different ways. It is conceivable that imatinib did not reach therapeutic levels in the plasma or tumor because of the concurrent use of enzyme-inducing antiepileptic drugs (EIAED). Alternatively, a high percentage of the glioblastoma multiforme patients included in those studies might not have expressed the relevant kinase targets or in those patients' tumors the target might not have been constitutively activated.

The present study was designed to address these questions and to examine the effect of imatinib in glioblastoma multiforme in a systematic fashion. First, by standardizing the antiepileptic regimen and using the non-EIAED valproic acid, steady plasma levels of imatinib were ensured and when measured, the plasma levels were comparable with those published in other tumors (20). In fact, in two important subsequent studies with the hydroxyurea/imatinib combination and the irinotecan/bevacizumab combination, the effect and toxicity of the regimens depended on the use of EIAED (21, 22).

Furthermore, because the ability of drugs to reach brain tumors is always questioned, imatinib was measured in tissue using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. Measuring the tissue concentration of a drug does not imply measuring exclusively intracellular levels. However, the evaluation of imatinib in tissue coupled with the posttreatment molecular and biochemical changes we have observed may constitute indirect evidence that the levels measured are at least partially intracellular. We examined pretreatment tumor vascularity in order to explain the difference in the range of average tissue and plasma levels but no relationship was found. Furthermore, we attempted to detect an effect of imatinib on proliferation, as reflected by changes in Ki67 expresssion immunohistochemically. No such effect was seen. Biochemical changes were detected, however, in some posttreatment specimens, compared with those obtained before treatment. Specifically, decreased phosphorylation of AKT and MAPK or increased expression of the p27 antiproliferation checkpoint protein was shown in 4 of 11 patients, suggestive of biochemical responses in these tumors. Notably, prior studies have indicated that the effect of imatinib on PDGFR in glioblastoma multiforme may be mediated via the AKT pathway (23). However, in the only glioblastoma multiforme showing high-level PDGFRB phosphorylation (patient 003/2), imatinib treatment had no apparent effect on PDGFRB activation, nor on activation of downstream signaling intermediates AKT and MAPK, and denaturing high-performance liquid chromatography and sequencing analyses (data not shown) did not reveal a definable *PDGFRB* genomic mutation. This observation suggests that activation of wild-type *PDGFRB* may not be adequately suppressed by imatinib treatment in glioblastoma multiforme, but it is also possible that this glioblastoma multiforme contained a novel *PDGFRB* mutation, which was resistant to imatinib therapy. Other investigators have detected effects of imatinib on MAPK in glioblastoma multiforme cell lines (24, 25), and it is widely believed that the potential target of imatinib in glioblastoma multiforme is PDGFR. Although several studies have examined the biochemical effect of imatinib on glioblastoma multiforme cell lines and some of them describe an effect on PDGFR (26), it is also well documented that certain gain-of-function mutations in *PDGFR*, *BCR-ABL*, and *KIT* are imatinib resistant (27, 28).

The oncogenic role of EGFR in glioblastoma multiforme in previous studies seems to be due to amplification and/or mutations in the *EGFR* gene (29, 30). Not surprisingly, we found two patients with high-level EGFR activation in this study, and each of those tumors had a known gain-of-function *EGFR* mutation (R108K and G598V), which was homozygous at the genomic level. In one report, 15% to 20% of glioblastoma patients showed clinical response to small-molecule EGFR

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

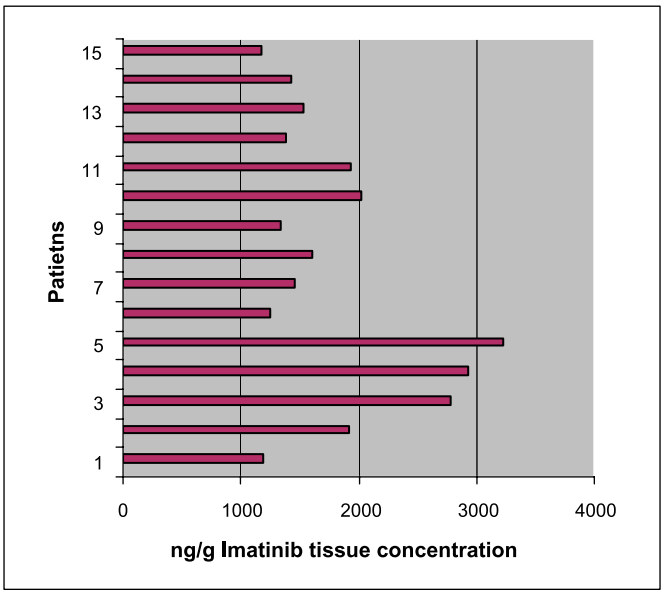

Fig. 1. Concentration of imatinib measured from glioblastoma tumor homogenates. Quantitative analysis of imatinib from 15 patient samples. The measurements range between 1,180 and 3,323 ng/g of tumor tissue with a median value of 1,530 ng/g. The average SD is 1%. Average values for triplicate measurements of the samples are presented.

kinase inhibitors (31). The persistence of high-level EGFR activation in posttreatment samples from these two patients is in keeping with the oncogenic *EGFR* mechanism, which may afford the cells a selective advantage to thrive during imatinib therapy.

It is well known that glioblastoma multiforme cells are very heterogeneous at the molecular level, with each abnormality appearing in low frequencies. Those abnormalities differentially activate and silence relevant signaling pathways. Thus, it may be that a particular target (namely PDGFRB, EGFR, AKT, or MAPK) may be more important in some patients than in others (32, 33). Furthermore, multiple signaling pathways may be activated concurrently and this redundancy may contribute to the resistance to single agents. For this reason, it is also possible that the biochemical evidence for AKT and MAPK inhibition, seen in two patients herein, represented biologic heterogeneity in the specimens analyzed before and after imatinib, rather than being a direct consequence of imatinib-mediated kinase inhibition. In a recent publication (34) the combination of erlotinib with imatinib and another receptor tyrosine kinase inhibitor, SU11274, on glioblastoma multiforme xenografts and cell lines enabled more effective target inhibition than the use of any of these agents alone.

There are several limitations in the present study. The short duration of treatment and the small number of patients precluded us from detecting a clinical (radiologic) effect that could in turn be correlated with the biochemical and protein effect for a more firm result, but because temozolomide and radiotherapy constitute standard therapy, it would have been unethical to delay this treatment.

Another consideration was the inherent extensive necrosis in brain tumors. Stereotactic biopsy specimens are small and the presence of necrosis results in even less tissue available for analysis. Additionally, necrosis hinders the assessment of response on magnetic resonance imaging, thus obstructing the correlation of clinical response with biochemical and protein changes. In fact, we attempted to evaluate the effect of imatinib on a proteomic level as well. Unfortunately, although peptide changes in pretreatment and posttreatment specimens were observed, the identification of the peptides was hindered by the lack of adequate tissue specimens, thus limiting the value of these observations. We feel it would be useful to pursue this type of proteomic investigation in the future.

In this study we observed a lower-than-expected overall survival, although two thirds of our patients did have standard temozolomide and radiotherapy after protocol completion, i.e., with a 2- to 4-week delay. One explanation for the short overall survival is that the surgeons opted to enroll patients with larger and thus particularly aggressive tumors, as those were less likely

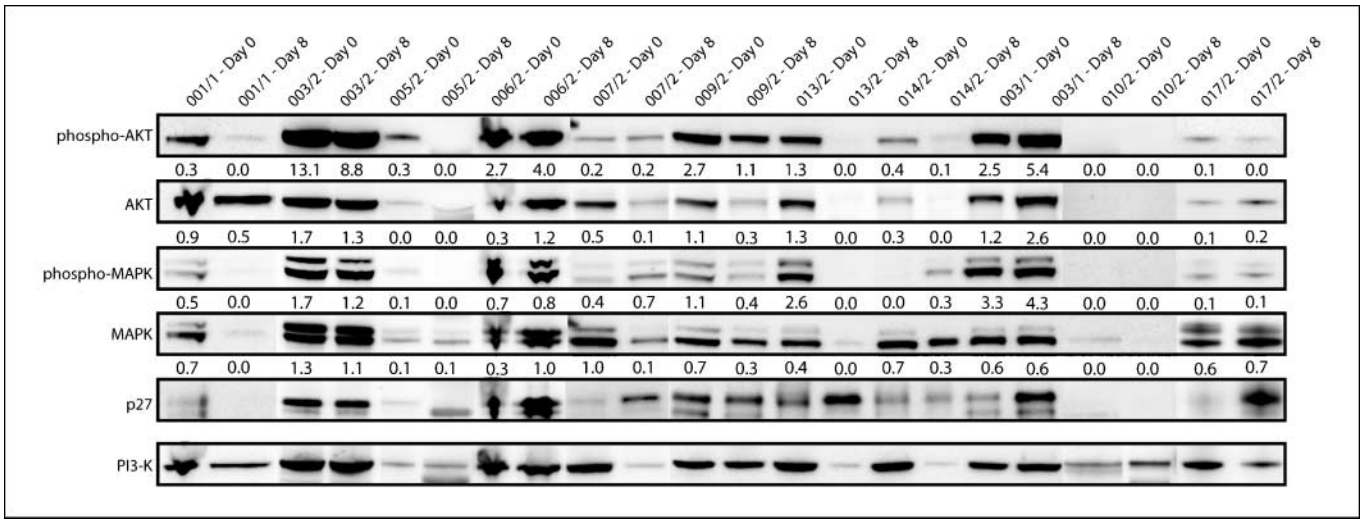

Fig. 2. Imatinib effects on AKT and MAPK activation, and p27 expression. PI3-K serves as a loading control, and quantitation of activated and total AKT and MAPK (numbers) was done in relationship to an identical internal standard on each blot. Imatinib treatment was associated with reduction in per molecule AKT and MAPK phosphorylation in GBM 001/1 and 009/2, respectively. Imatinib treatment was associated with increased p27 expression in GBM 007/2, 013/2, 003/1, and 017/2.

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

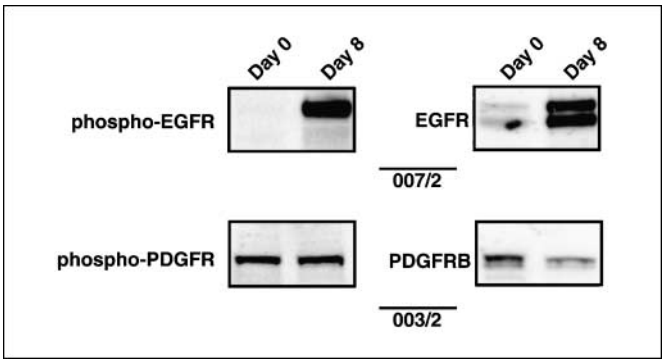

Fig. 3. EGFR and PDGFRB were strongly activated in one patient each.

to benefit from the standard approach. This seems consistent with the fact that almost half of the patients had a PS of 2 and almost half of the patients were not deemed eligible for definitive surgery on day 8, and thus proceeded with a second biopsy only. Furthermore, a full third of the patients did not receive standard therapy after protocol completion. In fact, the published literature supports the fact that older patients with poor PS that have not been debulked carry a worse prognosis (35).

However, this study is unique in its kind in glioblastoma multiforme because it examines the effect of a targeted agent in this tumor in a systematic way. In fact, in a recently published phase II study of imatinib in patients with recurrent gliomas, the authors point out the need for this type of investigation of drug penetration and target inhibition (36).

Overall this study provides proof of principle that rational trial design is feasible in gliomas and that the application of advanced technology can generate important data for a better understanding of the mechanism of action of targeted agents. Although there is extensive basic research in this area, we found that there is a paucity of translational studies. The importance of different mutations in glioma should be identified so that targeted agents are used accordingly. Although this study confirms the presence of imatinib in glioblastoma multiforme tissue and shows its molecular effects in a subset of patients, our results do not suggest a benefit from the use of the drug as a single agent in unselected glioblastoma multiforme patient populations. Instead, our results would suggest that the role of imatinib in glioblastoma multiforme needs to be elucidated further using a longer schedule, correlative science studies, and functional imaging.

Disclosure of Potential Conflicts of Interest

T. Kalebic, employment, Novartis Pharmaceuticals. J. Norris, research funds, Novartis Pharmaceuticals. Laboratory expenses of reagents or other consumables were paid by the Hellenic Cooperative Oncology Group (a nonprofit organization). No co-author was personally supported by HeCOG.

Acknowledgments

We thank Dr. Michael C. Heinrich of Oregon Health and Science University Cancer Institute in Portland, OR, for the genotyping studies on the glioblastoma multiformes with high EGFR and PDGFR activation, Mrs. Georgia Vourli for the statistical support, and Mrs. Maria Moschoni for secretarial support.

References

1. Newton HB. Molecular neuro-oncology and development of targeted therapeutic strategies for brain tumors. Part 1: growth factor and Ras signaling pathways. Expert Rev Anticancer Ther 2003;3:595–614.
2. Holdhoff M, Kreuzer KA, Appelt C, et al. Imatinib mesylate radiosensitizes human glioblastoma cells through inhibition of platelet-derived growth factor receptor. Blood Cells Mol Dis 2005;34:181–5.
3. Omuro AMP, Faivre S, Raymond E. Lessons learned in the development of targeted therapy for malignant gliomas. Mol Cancer Ther 2007;6:1909–19.
4. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001;344:1031–7.
5. Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med 2001;344:1052–6.
6. Labropoulos SV, Razis ED. Imatinib in the treatment of dermatofibrosarcoma protuberans. Biologics 2007;1:347–53.
7. Sihto H, Tynninen O, Butzow R, Saarialho-Kere U, Joensuu H. Endothelial cell KIT expression in human tumors. J Pathol 2007;211:481–8.
8. Hagerstrand D, Hesselager G, Achterberg S, et al. Characterization of an imatinib-sensitive subset of high-grade human glioma cultures. Oncogene 2006;25:4913–22.
9. Oertel S, Krempien R, Lindel K, et al. Human glioblastoma and carcinoma xenograft tumors treated by combined radiation and imatinib (Gleevec). Strahlenther Onkol 2006;7:400–7.
10. Pollack IF, Jakacki RI, Blaney SM, et al. Phase I trial of imatinib in children with newly diagnosed brainstem and recurrent malignant gliomas: A Pediatric Brain Tumor Consortium report. Neuro Oncol 2007;9:145–60.
11. Wen PY, Yung WKA, Lamborn KR, et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99–08. Clin Cancer Res 2006;12:4899–907.
12. Reardon DA, Egorin MJ, Quinn JA, et al. Phase II study of imatinib mesylate plus hydroxyurea in adults with recurrent glioblastoma multiforme. J Clin Oncol 2005;23:9359–68.
13. Guetens G, Prenen H, De Boeck G, et al. Imatinib tumor tissue analysis by measurement of sediment and by liquid chromatography tandem mass spectrometry. J Sep Sci 2006;29:453–9.
14. Fountzilas G, Karkavelas G, Kalogera-Fountzila A, et al. Post-operative combined radiation and chemotherapy with temozolomide and irinotecan in patients with high-grade astrocytic tumors. A phase II study with biomarker evaluation. Anticancer Res 2006;26:4675–86.
15. Weidner N. Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol 1995;147:9–19.
16. Titier K, Picard S, Ducint D, et al. Quantification of imatinib in human plasma by high-performance liquid chromatography-tandem mass spectrometry. Ther Drug Monit 2005;27:634–40.
17. Judson I, Ma P, Peng B, et al. Imatinib pharmacokinetics in patients with gastrointestinal stromal tumour: a retrospective population pharmacokinetic study over time. EORTC Soft Tissue and Bone Sarcoma Group. Cancer Chemother Pharmacol 2005;55:379–86.
18. Rubin BP, Singer S, Tsao C, et al. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 2001;15,61:8118–21.
19. Duensing A, Medeiros F, McConarty B, et al. Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). Oncogene 2004;13:3999–4006.
20. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 2005;44:879–94.
21. Desjardins A, Quinn JA, Vredenburgh JJ, et al. Phase II study of imatinib mesylate and hydroxyurea for recurrent grade III malignant gliomas. J Neurooncol 2007;83:53–60.
22. Vredenburgh JJ, Desjardins A, Herndon JE II, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 2007;25:4722–9.
23. Servidei T, Riccardi A, Sanguinetti M, Dominici C, Riccardi R. Increased sensitivity to the platelet-derived growth factor (PDGF) receptor inhibitor STI571 in chemoresistant glioma cells is associated with enhanced PDGF-BB-mediated signaling and sti571-induced Akt inactivation. J Cell Physiol 2006;208:220–8.
24. Quick QA, Gewirtz DA. Enhancement of radiation sensitivity, delay of proliferative recovery after radiation and abrogation of MAPK (p44/42) signaling by imatinib in glioblastoma cells. Int J Oncol 2006;29:407–12.
25. Gross D, Bernhardt G, Buschauer A. Platelet-derived growth factor receptor independent proliferation of human glioblastoma cells: selective tyrosine kinase inhibitors lack antiproliferative

Published OnlineFirst September 29, 2009; DOI: 10.1158/1078-0432.CCR-08-1867

activity. J Cancer Res Clin Oncol 2006;132:589–99.

26. Kilic T, Alberta JA, Zdunek PR, et al. Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class. Cancer Res 2000;60:5143–50.

27. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347:472–80.

28. Nardi V, Azam M, Daley GQ. Mechanisms and implications of imatinib resistance mutations in BCR-ABL. Review. Curr Opin Hematol 2004;11:35–43.

29. Libermann TA, Nusbaum HR, Razon N, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. Nature 1985;313:144–7.

30. Lee JC, Vivanco I, Beroukhim R, et al. Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain. PLoS Med 2006;3:485.

31. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2005;353:2012–24.

32. Habeler C, Gelpi E, Marosi C, et al. Immunohistochemical analysis of platelet-derived growth factor receptor-a, -β, c-kit, c-abl, and arg proteins in glioblastoma: possible implications for patient selection for imatinib mesylate therapy. J Neurooncol 2006;76:105–9.

33. Stupp R, Hegi ME, van den Bent MJ, et al. Changing paradigms-an update on the multidisciplinary of management of malignant glioma. Oncologist 2006;11:165–80.

34. Strommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science 2007;318:287–90.

35. Carson KA, Grossman SA, Fisher JD, Shaw EG. Prognostic factors for survival in adult patients with recurrent glioma enrolled onto the new approaches to brain tumor therapy CNS consortium phase I and II clinical trials. J Clin Oncol 2007;25:2601–6.

36. Raymond E, Brandes AA, Dittrich C, et al. Phase II study of imatinib in patients with recurrent gliomas of various histologies: a European Organisation for Research and Treatment of Cancer Brain Tumor Group Study. J Clin Oncol 2008;26:4659–65.

AACR American Association for Cancer Research

Clinical Cancer Research

Phase II Study of Neoadjuvant Imatinib in Glioblastoma: Evaluation of Clinical and Molecular Effects of the Treatment

Evangelia Razis, Panayotis Selviaridis, Stephanos Labropoulos, et al.

Clin Cancer Res 2009;15:6258-6266. Published OnlineFirst September 29, 2009.