

## Phase I and Correlative Biology Study of Cilengitide in Patients With Recurrent Malignant Glioma

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### ABSTRACT

#### Purpose

This multi-institutional phase I trial was designed to determine the maximum-tolerated dose (MTD) of cilengitide (EMD 121974) and to evaluate the use of perfusion magnetic resonance imaging (MRI) in patients with recurrent malignant glioma.

#### Patients and Methods

Patients received cilengitide twice weekly on a continuous basis. A treatment cycle was defined as 4 weeks. Treatment-related dose-limiting toxicity (DLT) was defined as any grade 3 or 4 nonhematologic toxicity or grade 4 hematologic toxicity of any duration.

#### Results

A total of 51 patients were enrolled in cohorts of six patients to doses of 120, 240, 360, 480, 600, 1,200, 1,800, and 2,400 mg/m<sup>2</sup> administered as a twice weekly intravenous infusion. Three patients progressed early and were inevaluable for toxicity assessment. The DLTs observed were one thrombosis (120 mg/m<sup>2</sup>), one grade 4 joint and bone pain (480 mg/m<sup>2</sup>), one thrombocytopenia (600 mg/m<sup>2</sup>) and one anorexia, hypoglycemia, and hyponatremia (800 mg/m<sup>2</sup>). The MTD was not reached. Two patients demonstrated complete response, three patients had partial response, and four patients had stable disease. Perfusion MRI revealed a significant relationship between the change in tumor relative cerebral blood flow (rCBF) from baseline and area under the plasma concentration versus time curve after 16 weeks of therapy.

#### Conclusion

Cilengitide is well tolerated to doses of 2,400 mg/m<sup>2</sup>, durable complete and partial responses were seen in this phase I study, and clinical response appears related to rCBF changes.

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### INTRODUCTION

Malignant primary brain cancer affects approximately 18,400 individuals in the United States a year.<sup>1</sup> Treatment has relied on surgical resection and radiation therapy with emerging roles for chemotherapy.<sup>2</sup> Unfortunately, survival for the most aggressive malignant glioma, glioblastoma multiforme, remains little changed during the past several decades.<sup>3</sup>

Malignant gliomas are intensely angiogenic and have the added detrimental behaviors of invasion and proliferation within the confines of the CNS.<sup>4-6</sup> The role of cell surface adhesion molecules has been identified as an important contributor to tumorigenesis in gliomas. Among these molecules, the integrins have a unique role, particularly in the development and progression of cancers of the nervous system.

Integrins are a class of cell surface adhesion molecules important in many cellular behaviors, including proliferation, survival, and migration.<sup>7</sup> In glioma samples, the integrins  $\alpha v \beta 3$  and  $\alpha v \beta 5$  are expressed by both the tumor-associated vasculature and tumor cells.<sup>8,9</sup> Integrin signaling is mediated through interactions with the arginine-glycine-aspartic acid peptide sequence present in select extracellular matrix proteins.<sup>10</sup> A role for the integrins  $\alpha v \beta 3$  and  $\alpha v \beta 5$  in glioma angiogenesis is supported by expression on activated endothelial cells (EC) within the tumor compared with normal brain EC<sup>9</sup> enrichment of the CNS with the integrin  $\alpha v \beta 3$  and  $\alpha v \beta 5$  ligand, vitronectin, and fibronectin.<sup>11,12</sup> In addition, it's supported by disruption of the integrin-ligand interaction both in vitro and in vivo by neutralizing antibodies or peptidomimetic compounds promoting vascular

regression.<sup>13</sup> Thus, this class of integrins is a logical therapeutic target in malignant glioma.

Cilengitide (EMD 121974, cycloL-Arg-Gly-L-Asp-D-Phe-N [Me] L-Val; Merck KGaA, Darmstadt, Germany) is a cyclic arginine-glycine-aspartic containing peptide that binds to  $\alpha\beta3$  and  $\alpha\beta5$  with nanomolar affinity. In cell adhesion assays, it inhibited both  $\alpha\beta3$  and  $\alpha\beta5$  mediated cell adhesion with  $IC_{50}$  values in the low micromolar range and inhibited angiogenesis in the chick chorioallantoic membrane assay and rabbit cornea assay.<sup>14</sup>

The rationale for the study of integrin antagonists in the setting of primary brain malignancies is thus provided. The present study was undertaken to determine the toxicities and maximum-tolerated dose (MTD) of the integrin antagonist cilengitide in patients with recurrent malignant gliomas.

## PATIENTS AND METHODS

This study was sponsored by the Cancer Therapy Evaluation Program at the National Cancer Institute (Bethesda, MD) and conducted by the New Approaches to Brain Tumor Therapy CNS Consortium (www.nabtt.org for participating institutions; Baltimore, MD). The protocol was reviewed and approved by the institutional review board at each participating institution and all patients signed informed consent.

Patients eligible for enrollment met the following criteria: 18 years or older; histologically proven malignant glioma; measurable progressive or recurrent tumor by magnetic resonance imaging (MRI); recovered from toxicities of previous therapies; maintained on a stable dose of corticosteroids for 5 or more days; not had more than two prior chemotherapy regimens; Karnofsky performance status of 60% or more; adequate hematologic, renal, and hepatic function; and capable of providing informed consent.

### Treatment Plan

This study was designed as an open-label, single-arm phase I trial to evaluate the toxicities and determine the MTD of cilengitide in patients with recurrent malignant glioma. Patients were infused intravenously with the study drug during a 1-hour period on a twice-a-week schedule with a minimum of 72 hours between infusions for 4 weeks. This 4-week interval was considered a treatment cycle. The cohort size was six evaluable patients. The starting cohort received cilengitide at a dose of 120 mg/m<sup>2</sup>, and protocol-defined dose increments were 120 mg/m<sup>2</sup>, up to a dose level of 600 mg/m<sup>2</sup>, then additional escalations to 1,200, 1,800, and 2,400 mg/m<sup>2</sup>. The starting dose and schedule were selected based on animal studies that indicated antitumor activity with a pulsed treatment schedule that obtained peak plasma concentrations of 11 to 13  $\mu$ g/mL and human studies that indicated a maximum concentration ( $C_{max}$ ) of 11 to 13  $\mu$ g/mL was obtained with dose levels of 120 mg/m<sup>2</sup>.<sup>15</sup> The MTD was defined as the dose level below the dose that induced dose-limiting toxicities (DLTs) in more than two of six patients or more than four of 12 patients in a cohort. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 3. Patients underwent evaluation of measurable disease by cranial MR every 8 weeks of treatment.

### Pharmacokinetics

For the cilengitide pharmacokinetic (PK) analysis, blood samples were collected in 7 mL green-top (heparinized) tubes, centrifuged at 1,000 g for 10 minutes to separate plasma, and stored at  $-70^{\circ}\text{C}$ . The samples were taken at the following time points: immediately before infusion, 0.5, 1, 1.5, 2, 3, 4, 8, and 24 hours after start of infusion on study day 1. Sample analysis was carried out using high-performance liquid chromatography-tandem mass spectrometry (Triple Quadrupole MS-Detector API 3000; Applied Biosystems, Foster City, CA). Calculation of pharmacokinetic PK parameters was performed at Merck KGaA (Darmstadt, Germany) by noncompartmental analysis using Kinetica version 4.1.1 (Thermo Scientific, Waltham, MA). For each patient the  $C_{max}$  and time to maximum concentration ( $t_{max}$ ) were the observed values. The area

under the plasma concentration-time curve (AUC) was calculated by the log-linear trapezoidal rule and extrapolated to infinity by linear regression analysis. The total body clearance of drug from plasma (CL) was calculated as dose/AUC. The apparent volume of distribution at steady-state ( $V_{ss}$ ) was calculated as dose  $\times$  AUMC/AUC<sup>2</sup> - [T/2], where AUMC is the area under the first moment curve to infinity and T the duration of the infusion. The apparent terminal half-life ( $t_{1/2}$ ) was calculated by dividing log<sub>2</sub> by the elimination rate constant ( $\lambda_z$ ), estimated by linear regression of the final data points of the linear portion of the log-linear concentration-time plot.

### Pharmacodynamic Analysis

The post processing and perfusion analysis was performed using the MEDx software version 3.4.2 (Sensor Systems, Sterling, VA) running a Sun Blade 1000 workstation with Solaris 8 Operating System (Sun Microsystems, Palo Alto, CA). The methodology and quality control measures have been previously reported.<sup>16</sup> Relative cerebral blood volume (rCBV) and relative cerebral blood flow (rCBF) determined at baseline and after 8 and 16 weeks of treatment were correlated with PK parameters and the clinical outcome, respectively.

### Correlative Biology Studies

**Perfusion MRI.** A total of 21 patients (41%) were imaged multiple times. The other patients had only a baseline scan. The perfusion imaging sequences (dynamic contrast susceptibility), included a baseline scan before first administration of cilengitide and follow-up perfusion scans performed after every 8 weeks of treatment with cilengitide.

The Gradient Recalled Echo-Planar Imaging sequence was used for acquiring functional perfusion data. This study used a repetition time of 1,900 msec and an echo time of 50 msec, accommodating a 20% variation in both repetition time and echo time to permit site-specific acquisition settings. A 24  $\times$  24-cm field of view was used and five to 10, depending on the size of the tumor, 6- to 8-mm axial slices passing through the center of the tumor were imaged during 40 to 65 functional time points. The contrast agent was injected with a power injector at a flow rate of 4 mL/sec.

### Statistical Considerations

Patient characteristics and toxicities were summarized using appropriate descriptive statistics. Survival time was calculated from the start of treatment until death from any cause, and surviving patients were censored at the time of last follow-up. The method of Kaplan-Meier<sup>17</sup> was used to estimate survival. CIs were calculated using standard methods. These analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC).

Spearman correlation coefficients were used to determine possible relationships between rCBF and rCBV differences from baseline to week 8 and week 16 versus dose of cilengitide,  $C_{max}$ , and AUC. Analysis of variance procedures were used to assess dose effects on rCBV and rCBF. Wilcoxon's signed rank test also was used to look at any change (over all doses) for differences in percentage of baseline rCBV and rCBF and time (8 or 16 weeks).

## RESULTS

### Patient Characteristics

Between August 2000 and February 2003, 51 patients were enrolled onto the study. Twenty-eight patients were taking enzyme-inducing anticonvulsant drugs (EIAD), nine patients were taking non-EIAD and 14 were not taking anticonvulsants. All patients had prior surgery and completed radiation therapy. There were 37 with glioblastoma multiforme, 11 anaplastic astrocytoma, one anaplastic oligodendroglioma, and two mixed anaplastic glioma. Two patients had no prior chemotherapies, 13 had one prior, and 35 had two prior chemotherapies. One patient included in the study did not meet eligibility criteria because he/she had three prior chemotherapies. Three patients progressed before completion of cycle one, were

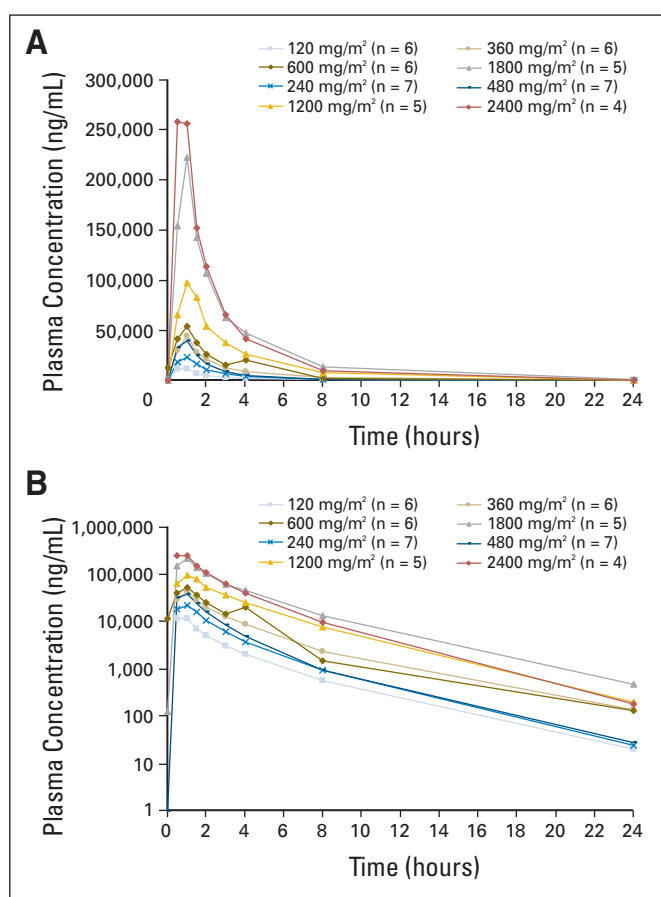
removed from treatment, and were replaced because they were inevaluable for DLT.

**Toxicity**

Patient infusion with cilengitide did not produce any acute toxicities. There were no more than one DLT in each cohort, which was insufficient to define a MTD. The DLTs occurred at the dose levels of 120, 480, 600, and 1,800 mg/m<sup>2</sup>. The event at 120 mg/m<sup>2</sup> was a thrombosis; at 480 mg/m<sup>2</sup> a grade 4 myalgia and arthralgia; at 600 mg/m<sup>2</sup> a grade 3 thrombocytopenia (platelet count of 37,000); at the time of off study; and at 1,800 mg/m<sup>2</sup> one patient experienced grade 3 anorexia, hyperglycemia, hypokalemia, and hyponatremia. The only hematologic toxicity was the thrombocytopenia, which was counted as a DLT because the platelet count was not repeated and the patient expired. There were no significant bleeding problems encountered with the administration of cilengitide. There were a total of 14 events with a grade of 3 or 4 and a relationship possibly to definitely related to cilengitide. These toxicities are listed in Table 1, with the corresponding dose level. Overall, these events were uncommon with 10 of the 14 occurring in less than 2% of patients. The more common, occurring in just 4% of patients, were hyperglycemia and hyponatremia, events typically associated with the underlying disease of malignant glioma and/or concurrent medications, such as corticosteroids or anticonvulsants. These were slightly concentrated in the upper two dose levels (eight of the 14). Overall, the study drug was well tolerated, and the majority of adverse events were attributed to the underlying disease.

**Pharmacokinetics**

The mean linear and semi-logarithmic plasma concentration-time profiles per dose level and the mean (standard deviation [SD]) PK parameters following single (day 1) infusion of cilengitide are shown in Figure 1 and Table 2, respectively. The relationship between dose and CL and V<sub>ss</sub> values, respectively, is illustrated in Figure 2. Mean values of cilengitide CL ranged between 5.9 and 12.1 L/h, which is low compared with hepatic and renal blood flow in man. The V<sub>ss</sub> was approximately 20 L, nominally equivalent to extra cellular fluid volume. The coefficient of variation for CL and V<sub>ss</sub> was not improved by dosing per body-surface area. Together, the small volume of distribution and low plasma clearance were manifested as a relatively short apparent terminal half-life of about 2.5 and 3 hours. No changes in CL,



**Fig 1.** The mean concentration time profiles for the various doses of cilengitide evaluated in the present study. (A) Linear scale; (B) log scale.

V<sub>ss</sub> and t<sub>1/2</sub> were observed between different doses, and C<sub>max</sub> and AUC values increased dose proportionally over the entire dose range indicating linear pharmacokinetics. All PK parameters were comparable with those reported in previous phase I studies of cilengitide.<sup>15</sup> Although the study was not stratified to test for differences in pharmacokinetics between patients taking P450 EIAD and those not, the dose

**Table 1.** Grade 3 or 4 Toxicities at Least Possibly Related to Cilengitide by Dose Level

Toxicity	Dose Level (mg/m <sup>2</sup> )								Patients (%; N = 51)
	120 (n = 6)	240 (n = 7)	360 (n = 6)	480 (n = 8)	600 (n = 6)	1,200 (n = 6)	1,800 (n = 6)	2,400 (n = 6)	
Anorexia	0	0	0	0	0	0	1	0	2
Arthralgia	0	0	0	1	0	0	0	0	2
Diarrhea	0	0	0	0	1	0	0	0	2
Hyperglycemia	0	0	0	1	0	0	1	0	4
Hypokalemia	0	0	0	0	0	0	1	0	2
Hyponatremia	0	0	0	0	0	0	1	1	4
Myalgia	0	0	0	1	0	0	0	0	2
Platelets	0	0	0	0	1	0	0	0	2
Seizure	0	0	0	0	0	0	0	1	2
ALT	1	0	0	0	0	0	0	0	2
SIADH	0	0	0	0	0	0	0	1	2
Uric acid	0	0	0	0	0	0	1	0	2

Abbreviation: SIADH, secretion of antidiuretic hormone.

**Table 2.** Overview of Pharmacokinetic Parameters of Cilengitide Following a 1-Hour IV Infusion

Dose (mg/m <sup>2</sup> )	C <sub>max</sub> (μg/mL)	t <sub>max</sub> * (h)	AUC <sub>0-∞</sub> (μg/mL*h)	t <sub>1/2</sub> (h)	CL (L/h/sqm)	CL (L/h)	V <sub>ss</sub> (L/sqm)	V <sub>ss</sub> (L)
120 (N = 6)								
Mean	12.9	1.0	30.8	3.01	4.23	8.22	10.2	20.2
SD	1.7		8.6	0.28	1.51	2.24	1.08	2.77
Range	11.2-16.1	0.5-1	16.9-42.5	2.69-3.4	2.83-7.12	6.12-12.5	9.1-12.1	15.9-24.1
CV, %					35.6	27.2	10.6	13.7
240 (N = 6)								
Mean	26.1	1.0	60.7	2.77	4.14	8.00	9.66	18.6
SD	3.7		13.1	0.16	1.1	2.82	1.14	3.84
Range	21.6-31.0	1-1	38.4-78.4	2.56-3.01	3.06-6.24	5.28-13.3	8.12-11.4	12.1-22.2
CV, %					26.5	35.3	11.8	20.7
360 (N = 6)								
Mean	44.7	1.0	128	3.13	3.02	5.90	9.37	18.3
SD	7.4		38.1	0.43	0.85	1.60	1.15	2.16
Range	35.2-56.9	1-1.1	81.7-195	2.71-3.79	1.85-4.41	3.43-7.90	8.35-11.6	15.3-20.8
CV, %					28.1	27.0	12.3	11.8
480 (N = 7)								
Mean	38.3	1.0	81.8	2.69	6.22	12.1	12.6	24.7
SD	9.1		21.1	0.31	1.65	2.81	2.69	5.96
Range	27.8-54.3	0.5-1.7	52.2-119	2.39-3.3	4.04-9.19	8.66-16.2	9.38-17.3	18.4-36.4
CV, %					26.5	23.2	21.4	24.1
600 (N = 2)								
Mean	62.6	1.0	139	3.23	4.65	7.98	10.6	18.9
SD	3.8		51.9	0.37	1.73	1.18	0.11	4.21
Range	59.9-65.3	1-1	102-176	2.97-3.5	3.42-5.87	7.15-8.81	10.5-10.6	16.0-21.9
CV, %					37.3	14.7	1.0	22.3
1,200 (N = 4)								
Mean	106	1.3	319	2.77	4.04	8.21	11.7	24.0
SD	35.3		96.6	0.39	1.26	2.46	3	6.86
Range	84.8-158	0.5-1.5	213-431	2.29-3.15	2.78-5.63	6.12-11.3	7.65-14.9	14.5-30.7
CV, %					31.2	29.9	25.7	28.5
1,800 (N = 5)								
Mean	222	1.0	578	2.82	3.29	6.45	8.81	17.4
SD	33.7		148	0.47	0.87	1.79	1.84	4.23
Range	168-256	1-1	426-749	2.48-3.63	2.4-4.22	4.59-8.99	6.88-11.3	12.1-21.6
CV, %					26.3	27.8	20.3	24.4
2,400 (N = 4)								
Mean	316	1.0	626	2.59	3.92	7.60	9.08	17.9
SD	128		95.9	0.14	0.71	1.54	3.56	7.81
Range	185-470	0.5-1	482-683	2.47-2.75	3.51-4.98	6.50-9.80	5.97-12.9	11.1-25.4
CV, %					18.1	20.3	39.2	43.7

Abbreviations: IV, intravenous; C<sub>max</sub>, maximum concentration; t<sub>max</sub>, time to maximum serum concentration; AUC, area under the plasma concentration-time curve; t<sub>1/2</sub>, terminal half-life; CL, mean systemic clearance; V<sub>ss</sub>, apparent volume of distribution at steady state; SD, standard deviation; CV, coefficient of variation.  
\*Median.

cohorts of six patients allowed such an evaluation to be made. We did not see a difference in CL, AUC, or C<sub>max</sub> based on concurrent administration of EIAD (data not shown).

### Response Assessment

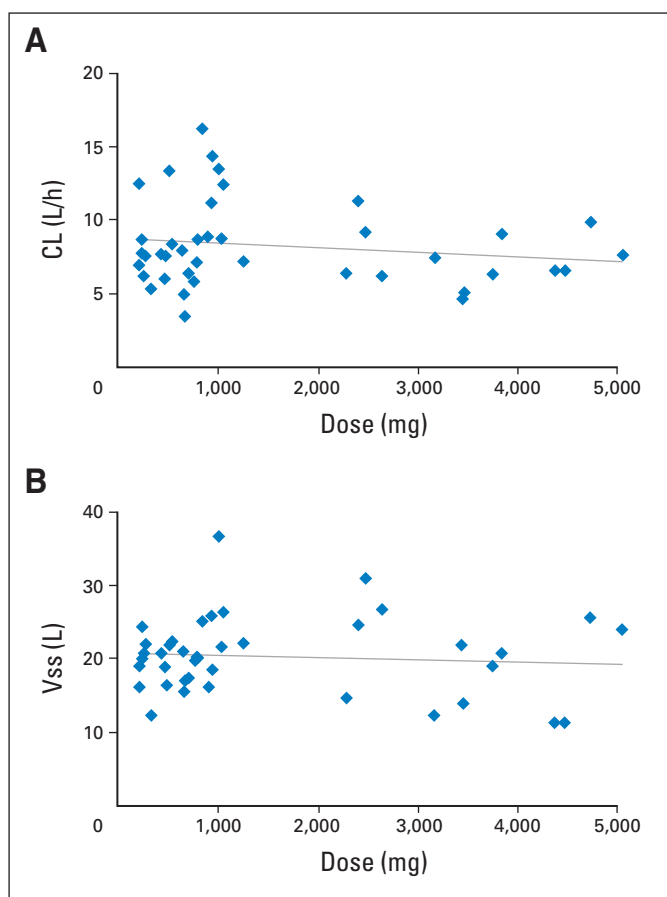
Of the 51 patients enrolled, there were a total of five objective responses. There were two complete responses in patients with anaplastic astrocytoma and a glioblastoma multiforme. These occurred at the 360 and 2,400 mg/m<sup>2</sup> dose levels. The complete response at 2,400 mg/m<sup>2</sup> occurred following the completion of the second cycle. Both patients were able to be tapered completely off corticosteroids and demonstrated improving functional status after the initiation of cilengitide therapy. Both patients voluntarily discontinued therapy after 24 and 12 months, respectively, and have been without clinical or radiographic evidence of recurrence at 29 and 15 months post-

treatment discontinuation, respectively. There were three patients that demonstrated a partial response with a mean duration of 9.3 months (range, 8-10 months). A total of 16 patients had a best response of stable disease with a mean duration of 5.4 months (range, 3 to 11 months).

Four (8%) of the 51 patients are alive after a minimum of 29 months of follow-up. The median survival time was 5.6 months (95% CI, 4.3 to 8.4 months). Kaplan-Meier estimates of survival stratified by dose level (< 600 and ≥ 600 mg/m<sup>2</sup>) did not differ (5.5 months for lower doses compared with 5.6 months for higher doses, log-rank *P* = .95).

### Correlative Biology End Point

**Perfusion MRI.** Dynamic contrast susceptibility-MRI was performed to quantify the changes in rCBV and rCBF. The changes from baseline were correlated with the dose of cilengitide as well as the PK



**Fig 2.** The relationship between various doses of cilengitide and the pharmacokinetic parameters (A) mean systemic clearance (CL) and (B) apparent volume of distribution at steady state ( $V_{ss}$ ).

parameters of  $C_{max}$  and AUC using Spearman rank correlation (Table 3). A negative relationship existed between the absolute change from baseline in rCBF and AUC at 16 weeks ( $r = -0.71$ ;  $P = .048$ ) suggesting that an increased target exposure was more likely to result in normalization of the rCBF. A meaningful correlation did not appear for percent change from baseline in either perfusion parameter at either time point nor for rCBF at 8 weeks. We also looked for percentage differences over all doses between baseline and week 8 or week 16 for the rCBV and rCBV; however, no significant difference was noted. Trends toward significance were present, but these comparisons were very underpowered to detect significant differences as would be the case in most phase I studies.

In Figure 3, we attempted to model the relationship between the PK parameters  $C_{max}$  and AUC and the pharmacodynamic (PD) parameters percentage of baseline rCBV and rCBF sorted by progressors and nonprogressors using the inhibitory maximum effect ( $E_{max}$ ) pharmacodynamic model. It was not possible to fit the data to the model in a meaningful way. However, a slight difference between progressors and nonprogressors could be seen from the data. After 8 weeks of cilengitide treatment, progressors showed more likely an increase in rCBV and rCBF compared with baseline values whereas nonprogressors stayed at least at the same level or even slightly below. All other data showed no obvious trend between the PK parameters and the PD parameters percent of baseline rCBV and rCBF, respectively.

**Table 3.** Spearman Rank Correlation Coefficients and Significance Values for Cilengitide Dose,  $C_{max}$ , and AUC Correlated With DCS-MRI rCBF and rCBV at Weeks 8 and 16

	Perfusion	Dose	$C_{max}$	AUC
<b>rCBF</b>				
Percentage of difference weeks 8-0, n = 25		0.16	0.14	0.12
Significance value		0.45	0.51	0.58
Weeks 8-0, n = 25		0.18	0.15	0.13
Significance value		0.40	0.47	0.54
Percentage of difference weeks 16-0, n = 8		-0.46	-0.31	-0.64
Significance value		0.25	0.46	0.09
Weeks 16-0, n = 8		-0.54	-0.40	-0.71
Significance value		0.17	0.33	0.05
<b>rCBV</b>				
Percentage of difference weeks 8-0, n = 25		0.08	0.08	0.04
Significance value		0.70	0.72	0.84
Weeks 8-0, n = 25		0.12	0.12	0.08
Significance value		0.56	0.58	0.72
Percentage of difference weeks 16-0, n = 8		-0.36	-0.14	-0.29
Significance value		0.39	0.74	0.49
Weeks 16-0, n = 8		-0.36	-0.14	-0.29
Significance value		0.39	0.74	0.49

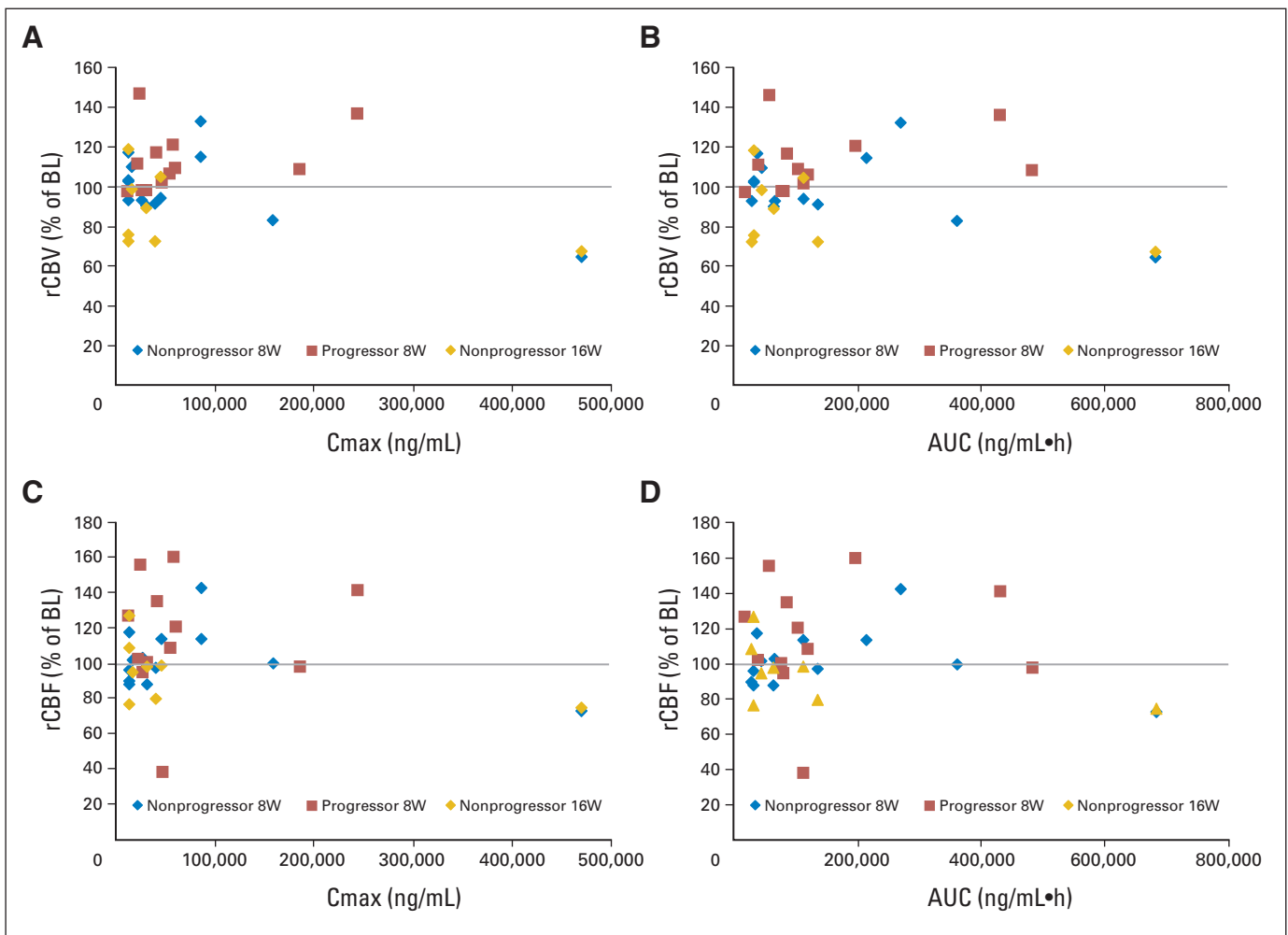
Abbreviations:  $C_{max}$ , maximum concentration; AUC, area under the plasma concentration-time curve; DSC-MRI, dynamic susceptibility contrast-magnetic resonance imaging; rCBF, relative cerebral blood flow; rCBV, relative cerebral blood volume.

## DISCUSSION

Cilengitide was shown to be well tolerated with limited toxicities when administered as a twice-a-week infusion, continuously in patients with recurrent malignant glioma. Toxicities were independent of dose, and there were insufficient DLTs to define a MTD. Most of the adverse events were mild and felt not to be attributed to cilengitide.

The pharmacokinetics of cilengitide in this study are comparable with those from other phase I studies of cilengitide.<sup>15</sup> The target  $C_{max}$  of 11 to 13  $\mu\text{g/mL}$  was achieved at the lowest dose evaluated, 120  $\text{mg/m}^2$ ; however, the duration of target exposure as reflected in the AUC was less than 5% of the highest dose administered, 2,400  $\text{mg/m}^2$ . The PK parameters for cilengitide were not affected by the concurrent administration of hepatic enzyme inducing anticonvulsants, and overall there was little variability in PK parameters among the patients as would be expected for an intravenously administered compound. Dosing per body-surface area had no impact on the coefficient of variation for CL and  $V_{ss}$ .

The correlative biology end points of this study support a role for DSC-MRI in the determination of a biologic response. As we have previously reported, a normalization of the perfusion parameters (rCBV and rCBF) was associated with the clinical response in a statistically significant manner.<sup>16</sup> In the present report, we were able to demonstrate a significant correlation or association between the PK parameter of AUC and change in the perfusion parameters of rCBF after 16 weeks of treatment. However, we were unable to fit the data to an inhibitory  $E_{max}$  model. An explanation for this lack of association may exist in the PD model chosen. The inhibitory  $E_{max}$  model has been successfully used in correlating the dose of the vascular



**Fig 3.** Relationship between pharmacokinetic and pharmacodynamic parameters: The percent of baseline, (A) relative cerebral blood volume (rCBV) for the maximum concentration ( $C_{max}$ ); (B) rCBV for area under the plasma concentration-time curve (AUC); (C) relative cerebral blood flow (rCBF) for  $C_{max}$ ; and (D) rCBF for AUC.

endothelial growth factor (VEGF)–receptor tyrosine kinase inhibitor, PTK787/ZK222584, the PK parameters, and the perfusion parameter ( $K_{trans}$ ) derived from dynamic contrast enhancement MRI.<sup>18,19</sup> The use of this model to study the relationship between a drug and its biologic response may best be served when that relationship approaches a linear behavior. For CNS cancers, that relationship may not be linear due to compartmentalization.

In addition, we should consider that this relationship might be made more complex in glioma when the target is potentially expressed by both the tumor-associated vasculature and the tumor itself. It is possible that in the cases where we documented clinical responses, the associated changes in perfusion parameters were not directly related to an antiangiogenic effect of cilengitide, but due to an indirect mechanism through an antitumor effect. For patients with significant target expression by the tumor, actual tumor responses could be expected as opposed to patients with predominant expression by tumor EC where stabilization may be the best response. This appears to be supported by our previous efforts documenting a statistically significant relationship between normalization of the perfusion parameters and clinical response<sup>16</sup> and the present study in which we report a statistically significant relationship between rCBF at 16 weeks and AUC (Table 3)

and the pharmacokinetic/pharmacodynamic plots in which the data suggest a difference after 8 weeks of treatment with progressors more likely to have an increase in rCBV and rCBF (Fig 3).

In summary, this is the first report of biologic activity by an integrin antagonist. Cilengitide demonstrated unexpected single-agent activity in patients with recurrent malignant glioma with limited toxicities. T2\**DSC*-MRI may be useful as a surrogate marker in the validation of clinical response; however, the utility to determine optimal biologic dose is limited in glioma patients using the PD inhibitory  $E_{max}$  model. The current study suggest that flat dosing is appropriate for future studies with a suggestion that higher doses (> 2,000 mg) are associated with clinical responses. Ongoing studies with cilengitide in glioma are examining tissue expression of the integrin targets as well as benefits of high versus low doses of the drug on survival in newly diagnosed and recurrent disease patients.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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## Appendix

The Appendix is included in the full-text version of this article, available online at [www.jco.org](http://www.jco.org). It is not included in the PDF version (via Adobe® Reader®).