A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma¹

Michael D. Prados,² S. Clifford Schold, Jr., Howard A. Fine, Kurt Jaeckle, Fred Hochberg, Laszlo Mechtler, Michael R. Fetell, Surasak Phuphanich, Lynn Feun, Todd J. Janus, Kathleen Ford, and William Graney

Department of Neurological Surgery, University of California San Francisco, San Francisco, CA 94143 (M.D.P.); Department of Neurology, University of Texas, Southwestern Medical Center, Dallas, TX 75390 (S.C.S.); Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA 02115 (H.A.F.); Department of Neurology, University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030 (K.J.); Department of Neurology, Massachusetts General Hospital, Boston, MA 02214 (F.H.); Department of Neurology, State University of New York at Buffalo, Buffalo, NY 14209 (L.M.); Department of Neurology, Columbia Presbyterian Medical Center, New York, NY 10032 (M.R.F.); Moffitt Cancer Center & Research Institute, Tampa, FL 33612 (S.P.); Division of Hematology/Oncology, University of Miami School of Medicine, Miami, FL 33136 (L.F.); Departments of Medical Oncology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242 (T.J.J.); Alkermes, Inc., Cambridge, MA 02139 (K.F., W.G.); USA

RMP-7, a bradykinin analog, temporarily increases the permeability of the blood-brain tumor barrier to chemotherapy drugs like carboplatin. We conducted a randomized,

controlled trial of carboplatin and RMP-7 versus carboplatin and placebo in patients with recurrent malignant glioma. The primary outcome measure was time to tumor progression (TTP). Adults with recurrent glioblastoma multiforme or anaplastic glioma were randomized in a 1:1 ratio to receive carboplatin and either RMP-7 or placebo. Radiation therapy had failed in all patients, and they may have received prior chemotherapy. Carboplatin (dosed to achieve an area under the curve of 5 mg/ml x time for patients who had received prior chemotherapy, or 7 mg/ml x time for those who had not) was given intravenously every 4 weeks, followed by intravenous infusion of either RMP-7 or placebo (300 ng/kg). TTP, tumor response, neuropsychological assessments, functional independence, and quality of life assessments were analyzed every 4 weeks. There were 122 patients enrolled, 62 in the RMP-7 and carboplatin group and 60 in the placebo and carboplatin group. Median TTP was 9.7 weeks (95% CI, 8.3–12.6 weeks) for the RMP-7 and carboplatin group and 8.0 weeks (95% CI, 7.4–12.6 weeks) for the placebo and car-

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² Address correspondence and reprint requests to Michael Prados, M.D., University of California San Francisco, 400 Parnassus Avenue, Room A808, San Francisco, CA 94143-0372 (PradosM@neurosurg.ucsf.edu).

³ Abbreviations used are as follows: AG, anaplastic glioma; AUC, area under the curve; GBM, glioblastoma multiforme; GFR, glomerular filtration rate; KPS, Karnofsky performance score; PFS, progression-free survival; RC, reliable change; SEM, standard error of measurement; TTP, time to tumor progression.

boplatin group. Median survival times were 26.9 weeks (95% CI, 21.3–37.6 weeks) for the RMP-7 group and 19.9 weeks (95% CI, 15.0–31.3 weeks) for the placebo group. No differences were noted for time to worsening of neuropsychological assessments, functional independence, or quality of life assessments. The use of RMP-7 had no effect on the pharmacokinetics or toxicity of carboplatin. At the dose and schedule used in this trial, RMP-7 did not improve the efficacy of carboplatin. Recent preclinical pharmacokinetic modeling of RMP-7 suggests that higher doses of RMP-7 may be required to increase carboplatin delivery to tumor. *Neuro-Oncology* 5, 96–103, 2003 (Posted to *Neuro-Oncology* [serial online], Doc. 02-034, February 19, 2003. URL <http://neuro-oncology.mc.duke.edu>)

Recurrent malignant glioma is particularly resistant to treatment. Surgery, radiation therapy, and chemotherapy have shown limited success. The blood-brain tumor barrier restricts entry of many potentially efficacious chemotherapeutic drugs. Essentially all hydrophilic substances and many lipophilic substances exceeding a molecular weight of 0.5 kDa are excluded (Pardridge, 1997). The blood-brain tumor barrier is made of vascular endothelial cells, with opposing membranes of adjacent capillary endothelial cells forming a tight junction that precludes entry of many agents. Although this barrier is leakier than the intact blood-brain barrier that is distant from the tumor, it nonetheless persists within the tumor vasculature and remains an obstacle to optimal drug delivery.

Several strategies have been developed to increase drug delivery across the blood-brain tumor barrier. One uses osmotic agents, which, when delivered by intracarotid infusion, shrink the endothelial cells and loosen the tight junctions temporarily to allow hydrophilic substances to diffuse into the brain (Kroll and Neuwelt, 1998). Because of the technical challenges and risk of brain and ocular injury, this approach is used in only a few centers. Another approach has been to develop drugs that can alter the permeability of the tight junction by modulating endogenous receptors expressed on the endothelial cells. Several drugs are now known to stimulate those receptors and, through a cascade of events, result in transient opening of these junctions. Bradykinin agonists have been studied extensively for this purpose (Raymond et al., 1986). Intracarotid infusions of bradykinin demonstrated that micromolar concentrations were sufficient to increase permeability of a range of compounds (Nomura et al., 1994). When bradykinin was given intravenously, however, the effect was achieved only when high concentrations were used, resulting in significant physiologic and endothelial toxicity.

Intravenous administration of the synthetic bradykinin agonist RMP-7 (Cereport, Alkermes, Inc., Cambridge, Massachusetts) made possible greater selectivity for the constitutively expressed B₂ receptor, a longer plasma half-life, and less toxicity, compared with bradykinin (Bartus et al., 1996a). In animal models, RMP-7 treatment increased the delivery of carboplatin into intracranial brain tumors (Matsukado et al., 1996). Carboplatin

is a water-soluble chemotherapy agent that is frequently used for patients with recurrent malignant glioma (Warnick et al., 1990; Yung et al., 1991). Initial single-arm, phase 2 studies were subsequently carried out using RMP-7 with carboplatin for patients with recurrent malignant glioma. These were suggestive of better survival and response rates compared to historical matched patient controls treated with carboplatin alone (Gregor et al., 1999). This preclinical and early clinical experience led us to design a multicenter, randomized, placebo-controlled study to compare the efficacy and safety of RMP-7 in combination with carboplatin versus placebo in combination with carboplatin for patients with recurrent malignant glioma.

Patients and Methods

Ten institutions across the United States participated in this multicenter trial. The University of California, San Francisco, provided central review of neuropathology and neuroimaging. A Safety Review Committee, composed of two clinicians and one biostatistician, who were not participants in the clinical study or employed by Alkermes, Inc., the study sponsor, assessed blinded safety data to confirm that safety parameters did not indicate an adverse effect of the combination of RMP-7 and carboplatin. This Committee met on a quarterly basis.

Eligibility

Patients were eligible for inclusion for this study if they were age 16 or older and had radiographically documented, measurable, recurrent glioblastoma multiforme (GBM)³ or anaplastic glioma (AG). Previous treatment had to have included surgery and radiation therapy and could have included prior chemotherapy. Patients had to have been receiving a stable dose of steroids for 2 weeks prior to study entry, and they had to have signed an informed consent form that had been approved by all 10 institutional review boards. Required pretreatment laboratory results had to include the following: an absolute neutrophil count >1500/mm³, platelet count >100,000/mm³, hemoglobin >8 g/dl, AST or ALT <2 times the institutional upper normal limits, creatinine <3.0 mg/dl, and total bilirubin <2.0 mg/dl.

Patients were excluded if they were pregnant or nursing. Women of childbearing potential were required to have had a negative pregnancy test prior to treatment and to agree to use a medically acceptable form of contraception throughout the study. Patients were excluded if they had received prior treatment with either carboplatin or cisplatin. The interval of prior radiation therapy or chemotherapy had to have been at least 4 weeks (6 weeks for prior nitrosourea chemotherapy) before treatment. The Karnofsky performance score (KPS) had to be 70 or greater, with a life expectancy of at least 2 months. There were no exclusions for the number of prior chemotherapy regimens used before enrollment in the trial or for the number of tumor recurrences prior to treatment.

Table 1. Neuropsychological assessments used in pretreatment evaluations and after every 4-week cycle

Cognitive Domain	Test	Time to Administer (min)
Attention span	Digit Span	5
Graphomotor speed	Digit Symbol	2
Memory	Hopkins Verbal Learning	5
Verbal fluency	Controlled Oral Word Association	3
Visual-Motor scanning	Trail Making Test Part A	3
Executive function	Trail Making Test Part B	5
Motor strength/dexterity	Hand Dynamometer, Grooved Pegboard	7
Quality of life	FACT-Brain Module (FACT-BR)	10
Functional status	Functional Independence Measure (FIM)	5
Total Time		45 min

Study Procedures

Pretreatment evaluations had to be completed within 2 weeks prior to beginning treatment. In addition to the laboratory tests described above, these evaluations included a baseline audiogram, measurement of the glomerular filtration rate (GFR) (used to calculate carboplatin dosing: see below), and a 9-item battery of neuropsychological tests (Table 1). A pretreatment MRI was required within 1 week of drug administration.

Patients were randomly assigned in a 1:1 ratio into 1 of 2 treatment groups: carboplatin and RMP-7 or carboplatin and placebo. A centrally generated randomization schedule for treatment assignments using permuted blocks within strata was used, with treatment group assignment balanced for tumor type (GBM or AG) and age group (<45 years; ≥45 years). At baseline, the pathologist at the local institution classified the tumor type for each subject enrolled. This assessment was reviewed for diagnostic confirmation by a central neuropathologist.

Treatment cycles were every 4 weeks. At the end of each cycle, patients underwent a physical and neurological examination, assessment of KPS, and the 9-item testing described above. Complete blood counts were obtained each week. A complete laboratory profile, including GFR, and audiograms were repeated every 8 weeks. Treatment consisted of carboplatin and either RMP-7 or placebo. Carboplatin dose was calculated according to the following formula:

$$\text{Dose (mg)} = \text{Target AUC (mg/ml} \times \text{minutes)} \times (\text{GFR} + 25)$$

The target area under the curve (AUC) was 7 for patients who had no prior chemotherapy (chemotherapy-naïve) and 5 for patients who had. Subsequent doses were given by using the baseline target AUC unless a dose reduction was required because of toxicity. Dose escalation to a higher target AUC was not permitted. The dose was recalculated every other cycle by using a repeat measurement of GFR. However, the GFR could be determined during any cycle if the serum creatinine rose more than 0.3 mg/dl from previous measurements.

The dose of RMP-7 or placebo was 300 ng/kg for all patients. Each was infused intravenously for 10 min by using a syringe infusion pump. The infusion began 5 min

before the end of a 45-min carboplatin infusion and continued for an additional 5 min after the carboplatin infusion ended. Clinical supplies of either RMP-7 or placebo were provided as double-blind material identified by treatment unit number. However, because RMP-7 may produce visible cutaneous flushing, revealing the identity of the treatment, the clinical staff who administered the drug were not allowed to participate in evaluating efficacy end points.

Patients continued to receive treatment every 4 weeks if their platelet count recovered to >100,000/mm³, their absolute neutrophil count recovered to >1500/mm³, and there were no unresolved grade 3 or 4 nonhematologic toxicities. A delay of 2 weeks was permitted for resolution of toxicity. If a patient developed grade 3 or greater hematologic toxicity, the carboplatin dose was adjusted downward. Treatment was discontinued if there was nonreversible grade 3 nonhematologic toxicity, grade 4 nonhematologic toxicity, or a delay in treatment longer than 2 weeks; if there were more than 2 episodes of febrile neutropenia; or if there was documented tumor progression.

Assessment of Efficacy

Subsequent MRI evaluations, which assessed tumor response and progression, were compared to the baseline MRI obtained 1 week before treatment began. Pre- and post-enhancement MRIs were conducted with gadolinium as the paramagnetic agent. A standard imaging schedule and standard procedures for obtaining the images were provided to each investigator site. The images, which were used to assess tumor size and volume, were forwarded to a central neuroradiologist for review of tumor volume. The neuroradiologist used software that permitted total tumor volume to be calculated by a 3-D tumor volume methodology that measured the slice area and thickness.

Treatment response was assessed both by the local investigator at the time of the patient's treatment cycle, and subsequently by the central neuroradiologist. The results of the central review were not used to determine whether patients received further treatment. The treating physician made that determination using the Macdonald criteria (Macdonald, 1990) as follows:

Complete Response	>95% reduction in tumor size with improved or stable neurological status and off all steroids
Partial Response	>50% and <95% reduction in tumor size with improved or stable neurological status with stable or reduced dose of steroids
Progressive Disease	>25% increase in tumor size, or new tumor, worsened neurological status with stable or increased steroid dose
Stable Disease	all other situations

The central neuroradiology review was used in the efficacy analysis, which was done when the clinical study was completed.

Statistical Methods

The sample size was based on the primary end point of TTP. A total of 80 patients were required to detect a doubling in the median TTP from an expected 8 weeks for the carboplatin-placebo group to 16 weeks for the carboplatin-RMP-7 group. The sample size was calculated for an alpha of 0.05 (2-tailed test) with a statistical power of 80% for detecting a doubling (or better) of TTP. The study accrual time was estimated to require 52 weeks, with a follow-up time of 24 weeks after the last patient was enrolled. This sample size would permit a difference in progression-free survival (PFS) rates of 30% to be detected with a power of >80%. For example, at 8 weeks, a carboplatin plus placebo PFS response of 50% compared with a carboplatin plus RMP-7 PFS response of 80% (difference of 30%) would be detected with 82% power.

Time to progression was defined as the time to an increase in tumor volume of $\geq 50\%$ (centrally reviewed) measured by MRI scans compared to the baseline MRI, or the time until frank clinical deterioration as determined by the investigator. The baseline MRI scan had to be performed within 14 days of the first drug infusion. Frank clinical deterioration was defined as the development of new or worsening neurological findings assessed by physical and neurological examinations. Although not used as the primary efficacy end point, MRI progression was also analyzed by using area measurements, which were based on the Macdonald criteria that required an increase of 25% or more from baseline.

The Kaplan-Meier survival distribution was estimated for each treatment group; between-group differences were compared by using the log-rank test. The survival time data were also analyzed by using the Cox proportional hazard regression model with covariates for baseline prognostic factors. All prognostic factors that were nonsignificant at the $\alpha = 0.2$ level were dropped from the analysis. A stratified log-rank analysis was used if the proportional hazards assumption required by the Cox model was not tenable. Statistical significance was declared when α was ≤ 0.05 ; all statistical tests were two-sided. The intent-to-treat population was defined as all subjects who received any study medication.

Secondary end points were the TTP from the small-

est tumor volume and changes in neuropsychological, functional independence, and quality of life assessments; response rates—time to response and duration of response; and survival. For each time-to-event for the secondary end points, the Kaplan-Meier survival distribution was estimated for each treatment group, and between-group differences were compared by using the log-rank test.

Patients were evaluated by a battery of neuropsychological assessments, quality of life testing using the FACT-BR test, and a measure of functional independence. The changes in test scores between the pretreatment baseline evaluation and subsequent assessments were determined by a reliable change (RC) index. This index is derived from the standard error of measurement (SEM) of each test. The SEM is calculated from the test-retest reliability (r) and the SD of test scores as follows:

$$\text{SEM} = \text{SD} (1-r)^{1/2}$$

The standard error of difference is then calculated as follows:

$$\text{SE}_{\text{diff}} = [2(\text{SEM}^2)]^{1/2}$$

A change in test scores from baseline to follow-up was considered significant if the RC was $\pm 1.64 (\text{SE}_{\text{diff}})$, a 90% confidence interval. The r and SD for each test were derived from information published in the test manuals, the original article describing the test, or texts describing the tests.

The following RC indices were calculated for each test:

Test	Score	$\pm$ RC
Digit Span	age-corrected scaled score	3
Digit Symbol	age-corrected scaled score	3
Hopkins Verbal Learning Test		
Total Recall	number across 3 trials	4
Recognition	hits minus false alarms	1
Controlled Word Fluency	age- and education-corrected scores	13
Trail Making Test part A	time in seconds to complete	12
Trail Making Test part B	time in seconds to complete	24
Grip Strength	kilograms	7
Grooved Pegboard	time in seconds to complete	12
FACT (total general score)	total score	11
FACT (brain subscale)	total score	19

The difference between the pretreatment baseline and each follow-up test was coded according to the above RC index as 1 (deterioration), 2 (no change), or 3 (improved). Frequency tables were used to show the percentage of patients in each treatment arm that showed meaningful losses in cognitive function or quality of life over the course of treatment. Kruskal-Wallis analyses of variance were computed to determine whether the distribution of

Table 2. Clinical characteristics of 121 patients treated with either RMP-7 and carboplatin or placebo and carboplatin*

Characteristic	RMP-7 Group	Placebo Group	P Value
Sex			0.354
Male	39 (64%)	32 (53%)	
Female	22 (36%)	28 (47%)	
Age (years)			0.902
Mean	49.9	49.5	
Range	22–81	20–75	
Baseline Tumor Volume (mm ³)			0.140
Mean	36,151.02	47,804.42	
Range	567.55–150,173	690.40–248,344.90	
Baseline KPS*			
Median	80%	80%	
Range	70–100	50–100	

*KPS = Karnofsky performance score.

RC indices differed between the two treatment arms. Analysis of group data, using a repeated measures multivariate analysis of variance, was used with the raw test scores, both within and between groups. Pearson product-moment correlations and *t*-tests were used to assess the interaction of the demographic and tumor-related variables on the outcome measures.

Results

A total of 122 patients were enrolled and randomized for treatment with RMP-7–carboplatin (62 patients) or placebo–carboplatin (60 patients). One patient randomized to receive RMP-7–carboplatin had elevated liver function at baseline and was not treated. Therefore, the analysis included 61 patients in the RMP-7–carboplatin group and 60 patients in the placebo–carboplatin group. The first patient was treated in March 1995, and the last patient completed treatment in January 1997. There were no statistically significant differences in baseline characteristics between the groups (Table 2). Tumor type was assessed locally by histology and was confirmed by central neuropathology review. The proportion of subjects with GBM was 40/61 (66%) for the RMP-7 group and 40/60 (67%) for the placebo group for both local histology and central review. Based on the local histology, the proportion of subjects with AG was 21/61 (34%) for the RMP-7 group and 20/60 (33%) for the placebo group. Based on the central review, the proportion of patients with AG was 18/61 (30%) in the RMP-7 group and 16/60 (27%) in the placebo group. Five of these 7 patients (1 in the RMP-7 group, 4 in the placebo group) were confirmed as less than grade 3; the remaining two subjects (both RMP-7) could not be confirmed because of a small sample size and uncertainty of grade due to background radiation effect. Overall, the percentage of patients who were chemotherapy-naïve was 23% in the RMP-7 treatment group and 30% in the placebo group. The number of tumor recurrences prior to the trial for each treatment group was generally comparable (Table 3).

The majority of patients in both treatment groups

Table 3. Tumor recurrences by treatment group in 121 patients studied

Treatment Group	Number of Recurrences (%)				
	1	2	3	4	5
RMP-7	45.9%	42.6%	11.5%	0	0
Placebo	38.3%	40.0%	13.3%	6.7%	1.7%

received no more than 2 cycles of treatment (56% in the RMP-7 group, 62% in the placebo group). Approximately one-third of patients received 3 to 6 cycles (34% in the RMP-7 group, 30% in the placebo group). The remaining patients received more than 6 cycles. Overall, patients in the RMP-7 group received a total of 187 cycles, and patients in the placebo group received 163 cycles. The primary reason for discontinuing treatment was tumor progression. Adverse events as the cause for discontinuation occurred in 7% of the RMP-7 group and in 5% of the placebo group. There were no deaths due to toxicity. The observed carboplatin AUC values were 95% (range 80–112%) and 87% (range 72–109%) of the values predicted for the placebo- and RMP-7-treated groups, respectively. Hematologic toxicity was the most common toxicity, which is typical for carboplatin. There were no differences in grade 3 or grade 4 hematologic toxicity between the groups. In addition, vasodilatation, headache, nausea, abdominal pain, and tachycardia, typical for a histaminelike reaction, were more frequently seen in the RMP-7 group. Table 4 summarizes toxicity for both groups.

Median TTP, calculated by using centrally reviewed tumor volume from baseline, was 9.7 weeks (95% CI, 8.3–12.6 weeks) in the RMP-7 group compared to 8.0 weeks (95% CI, 7.4–12.6 weeks) for the placebo group (Table 5). This was not statistically significant. The same was true whether comparing groups by histology (GBM or AG), or adjusting for other prognostic variables. When TTP was analyzed by using changes based upon tumor area (rather than volume), median TTP was 10.1 weeks for RMP-7 and 7.9 weeks for the placebo group. Thus, using area determination or volume determination

Table 4. Summary of statistically significant* adverse events: Toxicity difference between groups

Adverse Event/Toxicity	Treatment Group N (%)	
	RMP-7 (n = 61)	Placebo (n = 60)
Vasodilatation	48 (79%)	15 (25%)
Nausea	34 (56%)	17 (28%)
Headache	25 (41%)	12 (20%)
Asthenia	17 (28%)	6 (10%)
Abdominal pain	14 (23%)	1 (2%)
Tachycardia	13 (21%)	3 (5%)
Back pain	7 (11%)	0
Abnormal vision	4 (7%)	13 (22%)

*Statistically significant at $P \leq 0.05$, Fisher's exact test.

Table 5. Median time to tumor progression (weeks)*

Patient Population	Treatment Group	
	RMP-7 (95% CI) (n = 61)	Placebo (95% CI) (n = 60)
<i>All Patients</i>	9.7 (8.3–12.6)	8.0 (7.4–12.6)
Anaplastic glioma	10.1 (8.3–22.9)	8.6 (7.0–>24)
Glioblastoma multiforme	8.6 (7.9–11.6)	8.1 (5.9–16.1)
<i>All Patients by Previous Chemotherapy</i>		
Previous chemotherapy exposure	9.0 (7.9–11.0)	7.9 (5.9–9.7)
Chemotherapy-naïve	19.4 (8.6–>23.3)	12.6 (7.7–21.3)

* Median time to tumor progression was assessed by magnetic resonance images of tumor volume or frank clinical deterioration.

Table 6. Median and 6-month and 12-month survival of 121 patients studied

	Treatment Group	
	RMP-7	Placebo
<i>Median Survival in Weeks (95% CI)</i>	n = 61	n = 60
All Patients	26.9 (21.3, 37.6)	19.9 (15.0, 31.3)
Anaplastic Glioma	42.1 (21.3, 64.3)	24.7 (12.3, 50.6)
Glioblastoma multiforme	24.0 (17.4, 29.9)	21.1 (16.0, 32.1)
<i>6-Month and 12-Month Survival Rates (95% CI)</i>	Prior Chemotherapy Exposure	
6-month survival rate	51% (37%, 65%)	43% (28%, 58%)
12-month survival rate	22% (10%, 35%)	20% (8%, 33%)
	Chemotherapy-Naïve	
6-month survival rate	64% (39%, 89%)	39% (16%, 61%)
12-month survival rate	43% (17%, 69%)	28% (7%, 48%)

did not alter the primary end point analysis in this study. Response (according to the Macdonald criteria) was similar in the 2 groups. Among the RMP-7 group, there were 3 partial responders and no complete responders; among the placebo group, there were 4 partial responders and 1 complete responder. Median survival times and 6-month and 12-month survival rates are shown in Table 6. Although median survival time was longer in the RMP-7 group (26.9 weeks vs. 19.9 weeks), the differences were not statistically significant. Among patients with AG, median survival time was 42.1 weeks in the RMP-7 group and 24.7 weeks in the placebo group. For patients with GBM, median survival time was 24.0 weeks in the RMP-7 group and 21.1 weeks in placebo group. Chemotherapy-naïve patients treated with RMP-7 tended to have a longer median survival time (29.5 weeks vs. 21.1 weeks) and higher 6-month (64% vs. 39%) and 12-month (43% vs. 28%) survival rates than those receiving placebo, although these results are not statistically significant. There were no statistically significant differences between the treatment groups for time to worsening on the neuropsychological assessments, quality of life, and functional status (data not shown).

Discussion

This study was designed to detect a doubling of the TTP by using RMP-7 in combination with carboplatin, com-

pared to the TTP associated with placebo with carboplatin. Although encouraging trends were noted in many of the efficacy end points, none reached statistical significance. It is possible that these trends could be validated with a larger sample size, and by trying to detect a more modest improvement in efficacy. In a heavily pretreated patient population, it is of interest to see even a small difference in survival, as was noted in the AG group of patients (median survival of 42.1 weeks in the RMP-7-treated group compared to 24.7 weeks for the placebo-treated group). Likewise, it is of interest to note the encouraging trends in the chemotherapy-naïve group treated with RMP-7 and carboplatin. However, this trial, using the design noted, was a negative study. The blood-brain tumor barrier remains a formidable obstacle in the treatment of patients with malignant brain tumors. Drug delivery to tumor is difficult, and many chemotherapy agents that seem effective when tested in vitro will not translate into an effective therapeutic strategy when used in patients. Therefore, research designed to improve drug delivery is an important area of interest for neuro-oncology. Techniques that use intravenous agents to modify permeability and increase efficacy increase the possibility of greater use by physicians caring for these patients. The observation that specific endogenous receptors exist on the endothelial cell, and that they could be used to change permeability, enhanced the use of intravenous agents to modify the blood-brain tumor barrier. Bradykinin, in particular, was able to modulate the capillary endothelial

tight junction, thereby increasing blood-brain barrier permeability (Nomura et al., 1994; Raymond et al., 1986). However, the very high doses of intravenous bradykinin needed to cause this effect were toxic to the endothelial cells and caused physiologic stress to animals tested. Further experiments with intracarotid infusions of bradykinin showed that smaller doses were sufficient to increase permeability (Nomura et al., 1994). RMP-7, a bradykinin analog developed along these lines, has a longer plasma half-life than bradykinin and is selective for the constitutively expressed B₂ receptor on the endothelial cells forming the tight junction (Bartus et al., 1996a). Preclinical research confirmed that RMP-7 also increases permeability not only of the brain-tumor barrier but of the "normal" blood-brain barrier as well. In a rat glioma model, intracarotid infusion of RMP-7 was shown to increase the delivery of carboplatin more than 2-fold and to improve survival (Emerich et al., 1999). Intravenous use of RMP-7 was also effective in the rat glioma model but required higher doses than were needed with intracarotid infusions (Elliot et al., 1996). Carboplatin was chosen as the chemotherapy drug for these experiments because it was hydrophilic and had been shown to be modestly effective when used either intravenously or intra-arterially in patients with malignant glioma. It also has a demonstrated dose-escalation effect and had the potential to increase the cytotoxic effect against tumor if dose delivery were enhanced. Subsequent to these laboratory investigations, clinical trials were started that used intracarotid or intravenous infusions of RMP-7 along with carboplatin in patients with malignant glioma. Because single-arm phase 1 and phase 2 studies have had encouraging results (Cloughesy et al., 1999; Macdonald et al., 1990), we hoped that our randomized, placebo-controlled phase 2 trial would demonstrate efficacy of RMP-7 used with carboplatin.

Several lines of investigation have since been reported that suggest that the optimal therapeutic use of intravenous RMP-7 and carboplatin may require some modification of the schedule used in our study. Because of the transient nature of the change in permeability of the blood-brain tumor barrier, precise timing of both the receptor agonist and the chemotherapy agent are critical elements for success. For instance, it has been shown that the increase in permeability will persist only for minutes after the RMP-7 infusion has been completed, and that tachyphylaxis occurs with continuous dosing schedules (Bartus et al., 1996a,b). Ideally, the peak plasma level of carboplatin should coincide precisely with the time that the barrier is most permeable. In our study, the infusion of a 300-ng/kg dose of RMP-7 began 5 min before the end of a 45-min carboplatin infusion and continued for an additional 5 min after the carboplatin infusion ended.

In a series of experiments, Emerich et al. studied various dose-scheduling regimens of intravenous RMP-7

and carboplatin using a rat model of intracranial glioma (Emerich et al., 1999). Carboplatin pharmacokinetics plasma profiles were determined by using either a bolus injection or 15-min infusions (Emerich et al., 1999). C-labeled carboplatin was used to measure carboplatin uptake in the brain tumor relative to the timing of the RMP-7 infusion. RMP-7 increased concentration of carboplatin in the tumor by 75.5% when carboplatin was given as a bolus 5 min into the RMP-7 infusion. However, when both RMP-7 and carboplatin were given as 15-min co-infusions, no enhanced levels of carboplatin were noted. Although the AUC of carboplatin differed by only <20% with either a bolus injection or 15-min infusion, the C_{max} was higher and occurred earlier with the bolus injection. Thus, using a strategy to time the C_{max} for carboplatin with the maximal permeability effect resulted in greater tumor carboplatin concentrations. In addition, the timing of carboplatin infusion relative to the initiation of RMP-7 infusion was important. In these experiments, RMP-7 was given as a fixed 15-min infusion. When carboplatin was infused as a bolus 5 min into the RMP-7 infusion, carboplatin tumor levels increased 174% from baseline. When carboplatin was given 10 min into the RMP-7 infusion, carboplatin tumor levels increased 112.4% from baseline. However, when carboplatin was given 15 min from the start of the RMP-7 infusion, no increase in tumor carboplatin levels was found. Permeability was apparently restored because of tachyphylaxis.

Recently, Bartus et al. used a similar rat glioma model to study a dose-effect of RMP-7 and carboplatin (Bartus et al., 2000). They noted an increase in platinum levels within the brain tumors that persisted for at least 2 h, and that was particularly important in modulation of the blood-brain tumor barrier, which is normally impermeable to carboplatin. RMP-7 doses that produced plasma concentrations in the range of 25 to 50 nM resulted in a nearly 2-fold greater median survival time than controls (36 days vs. 19 days; $P < 0.01$). Lower doses of RMP-7 that achieved plasma concentrations in the range of 8 to 16 nM resulted in a median survival time of 29 days. When pharmacokinetic methods were used to model RMP-7 in human patients, an estimated dose range of 1200 to 1500 ng/kg was thought likely to reach these more optimal levels, a much higher dose than we used in our study.

In summary, carboplatin in combination with RMP-7 in the doses and schedules used in this trial was not successful in patients with recurrent malignant glioma. Higher doses of RMP-7, timed to coincide with the C_{max} of carboplatin, should be considered for further experimentation, to see if the best dose-schedule paradigm can improve survival. Our negative findings for RMP-7 and carboplatin should not preclude or deter the use of this novel strategy of drug delivery for other single agents or for multiple drugs.

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