

Survival of Patients With Newly Diagnosed Glioblastoma Multiforme Treated With RSR13 and Radiotherapy: Results of a Phase II New Approaches to Brain Tumor Therapy CNS Consortium Safety and Efficacy Study

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Purpose: The objectives of this phase II study were to determine survival, safety, pharmacokinetics (PK), and pharmacodynamics (PD) of 2,4-[[[3,5-dimethylanilino]carbonyl]methyl]phenoxy]-2-methylpropionic acid (RSR13, efaproxiral) 100 mg/kg per day administered with standard cranial radiotherapy (RT) for the treatment of glioblastoma multiforme (GBM). RSR13, a synthetic allosteric modifier of hemoglobin, is a radiation-enhancing agent that noncovalently binds to hemoglobin, reduces oxygen-binding affinity, and increases oxygen unloading to hypoxic tissue.

Patients and Methods: Fifty patients with newly diagnosed GBM (Karnofsky performance status \geq 60) were enrolled onto this multicenter phase II study. Patients received daily RSR13 100 mg/kg intravenously infused for 30 minutes immediately before cranial RT (60 Gy in 30 fractions). Supplemental oxygen

was given during RSR13 infusion and continued until after the RT treatment was completed. RT was given within 30 minutes of the end of RSR13 infusion. PK and PD determinations were performed.

Results: The median survival for the RSR13-treated patients was 12.3 months with 1-year and 18-month survival rates of 54% and 24%, respectively. Twenty-four percent of patients had greater than grade 2 toxicity, which was generally transient and self-limited. A significant PD effect on hemoglobin-oxygen binding affinity was demonstrated for most patients.

Conclusion: RSR13 (100 mg/kg) administered immediately before cranial RT is well tolerated and is pharmacodynamically active. Median survival in excess of 1 year is favorable.

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RSR13 (EFAPROXIRAL), 2,4-[[[3,5-dimethylanilino]carbonyl]methyl]phenoxy]-2-methylpropionic acid, is a synthetic allosteric modifier of hemoglobin with the potential to act as a radiation enhancing agent and chemosensitizer. Pre-clinical studies have demonstrated the ability of RSR13 to increase tumor oxygen delivery and to thereby enhance the response to both fractionated radiotherapy (RT) and some chemotherapy agents.¹⁻⁴ Glioblastoma multiforme (GBM) was selected as an initial tumor type for the study of this agent because this tumor is known to be hypoxic⁵ and relatively radioresistant.⁶⁻⁸ If RSR13 in combination with RT proves efficacious in GBM, it may also be beneficial in the therapy of other primary tumors. The New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium^{9,10} has previously reported the results of a phase I dose and schedule finding study of RSR13 that demonstrated that RSR13 100 mg/kg/d infused over 1 hour during a standard 6-week course of cranial RT was safe and pharmacodynamically active.¹¹ We now report the results of a study conducted by the NABTT CNS Consortium to estimate survival and safety in 50 patients with newly diagnosed GBM. This is the first study to report survival results for RSR13 used in the therapy of a primary tumor.

RSR13 is a synthetic allosteric modifier of hemoglobin that noncovalently binds to the central water cavity of the hemoglobin tetramer and stabilizes the deoxyhemoglobin state.¹²⁻¹⁴ As a result, hemoglobin-oxygen binding affinity

is reduced, leading to increased oxygen unloading to hypoxic tissues. Hypoxic cells, which are common in solid tumors, are known to be resistant to RT.¹⁵ Because RSR13 uses the substantial oxygen carrying capacity of hemoglobin, it has the potential to enhance tissue oxygenation to a greater degree than other approaches that have been tested in the past.^{1,16} With this approach, only molecular oxygen, not RSR13, needs to cross the blood-brain barrier and enter the tumor.

The results of a 50-patient safety and efficacy study of RSR13 100 mg/kg administered each day of standard

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cranial RT are reported here. To enhance the pharmacodynamic (PD) effect and to reduce the use of clinic resources, the RSR13 infusion time was reduced from the 60 minutes in the phase I dose-finding study to 30 minutes in this phase II study. RT consisted of a total dose of 60 Gy given in 30 treatments over 6 weeks. The primary objective of this trial was to estimate survival outcome with this therapy. Secondary objectives of this study included obtaining additional information about safety, pharmacokinetics (PK), and PD effects.

PATIENTS AND METHODS

Patients and Eligibility

Patients were required to have histologically confirmed supratentorial grade 4 astrocytoma (GBM) untreated except for biopsy/surgery and/or corticosteroids. Other eligibility criteria included age ≥ 18 , Karnofsky performance status ≥ 60 , arterial oxygen saturation (SaO_2) $\geq 90\%$ by pulse oximetry, WBC $\geq 2,000$ cells/ μL , hemoglobin ≥ 10 g/dL, platelet count $\geq 100,000$ cells/ μL , bilirubin ≤ 2.0 mg/dL, alkaline phosphatase and transaminases ≤ 3.0 times the upper limit of normal, and creatinine ≤ 2.0 mg/dL. Exclusion criteria included hemoglobinopathy, concurrent pulmonary conditions that might compromise oxygen loading unless pulmonary function tests could confirm that the forced vital capacity and forced expiratory volume in one second were $\geq 60\%$ of normal exercise, intercurrent illness that might interfere with protocol treatment, and concurrent malignancy unless disease-free ≥ 5 years (except for curably treated basal cell or squamous cell carcinoma of the skin or carcinoma-in-situ of the cervix). Women of child bearing potential were required to have a negative serum beta human chorionic gonadotropin pregnancy test, agree not to breast feed, and to use a standard contraceptive regimen. This National Cancer Institute/Cancer Therapy Evaluation Program and the institutional review boards of all participating institutions approved the protocol. Informed consent was obtained from all patients before participating in this study.

RSR13 Preparation

RSR13 was supplied by Allos Therapeutics, Inc, Denver, CO. RSR13 was formulated as a sterile solution of 2 g of RSR13 in 100 mL of 0.225% NaCl, with a resulting concentration of 20 mg/mL and an osmolality approximately equivalent to half-normal saline. Within 6 hours before drug administration, RSR13 was drawn from each 100-mL stock bottle and passed through a sterile filter directly into a sterile commercial infusion bag.

RSR13 Administration

RSR13 was administered by a central venous infusion device to avoid pain that is sometimes associated with peripheral infusion. Before each scheduled RSR13 administration, the SaO_2 was assessed by cutaneous pulse oximetry. If the SaO_2 was $\geq 90\%$, RSR13 was administered; if the SaO_2 was less than 90%, RSR13 was not given before RT treatment. To optimize tumor oxygen delivery and insure sufficient arterial oxygenation during the period when oxygen affinity was reduced by RSR13, patients received 4 L/min of supplemental oxygen via a nasal cannula beginning 5 minutes before the start of the drug administration. Patients were monitored with pulse oximetry,

and the oxygen flow rate was increased if the SaO_2 fell below 90%. Supplemental oxygen was discontinued and patients were discharged from the outpatient clinic only when their SaO_2 was $\geq 90\%$ on room air.

Vital signs were monitored before the infusion every 60 minutes until discharge, at discharge, and as clinically indicated. Pulse oximetry readings were recorded before supplemental oxygen, before the infusion while on oxygen, and every 30 minutes until discharge, at discharge, and as clinically appropriate. Patients were weighed weekly, and RSR13 dosing was recalculated if there was a 10% or greater change from the weight at which the dose was originally calculated.

RSR13 (100 mg/kg of body weight) was infused over 30 minutes at a constant rate via a volumetric infusion pump. If the infusion was interrupted for any reason, the infusion time was extended but was not to exceed 45 minutes, even if the entire dose of RSR13 had not been delivered. RT began within 30 minutes of the end of RSR13 infusion. RSR13 doses could be omitted for toxicity or logistic issues, but there was no provision for daily dose reduction. If RSR13 was not administered for any reason other than treatment related toxicity or hypoxemia, then daily RT treatment could be held and treatment with combined therapy resumed the next day. If RSR13 could not be administered as a result of any treatment-related toxicity or hypoxemia, the scheduled radiation was given as planned without RSR13.

RT

Standard cranial RT to a dose of 60 Gy in 2.0 Gy fractions for 30 fractions over 6 weeks was given. The initial field was treated to 46.0 Gy delivered to the tumor plus edema with a 2 cm margin if determined by magnetic resonance imaging (MRI) or 3 cm margin if determined by computed tomography (CT) scan. The conedown received an additional 14.0 Gy to the gross tumor or tumor bed with a 2.0 cm margin if determined by MRI and a 3.0 cm margin if determined by CT scan. If a three-dimensional treatment planning system was used, the dosimetric margin for the 100% isodose line was 1 cm if determined by MRI and 2 cm if determined by CT scan.

Toxicity Assessment

Patients were assessed for adverse events and toxicity during each treatment day and for 1 month after completion of RSR13-RT. The Radiation Therapy Oncology Group (RTOG) Acute Morbidity Criteria were used for radiation related toxicity. National Cancer Institute common toxicity criteria were used for laboratory abnormalities. Dose-limiting toxicity was defined as any grade 3 or 4 toxicity (except for grade 3 neurotoxicity responding to corticosteroids, diuresis, or anticonvulsants) thought to be possibly, probably, or definitely related to RSR13. Chemistry profile, complete blood count, prothrombin time/partial thromboplastin time, urinalysis, and anticonvulsant levels were performed pretreatment and weekly.

PK and PD Determinations

PK plasma and RBC drug concentrations and PD determinations were performed by representatives of Allos Therapeutics, Inc. Blood samples for PK and PD determinations were collected once each week. PD samples were analyzed for whole blood p50 determinations by multipoint tonometry. The p50 is the partial pressure of oxygen (PO_2) at which hemoglobin is 50% saturated. The targeted PD effect was a shift in p50 of approximately 10 mmHg. Samples for PK and PD determinations on the scheduled days were drawn before the infusion, after the infusion, and either 3 hours after the start of infusion or at the time of discharge from the clinic, whichever occurred first.

Table 1. Baseline Demographic and Clinical Characteristics

	RSR13 (n = 50)	9-AC (n = 50)	Paclitaxel (n = 33)	CI-980 (n = 39)
Sex, % male	64.0	72.0	63.6	64.1
Race, % white	98.0	96.0	93.9	94.9
Mean \pm SD age, years	58.0 \pm 10.3	56.8 \pm 12.1	58.2 \pm 7.9	62.2 \pm 10.8
Mean KPS \pm SD	86.0 \pm 12.0	88.1 \pm 10.3	87.9 \pm 13.4	85.8 \pm 12.4
Surgery type,* % resection	78.0	62.0	73.3	74.4

NOTE. Thirteen patients were missing KPS; 9-AC (n = 7), paclitaxel (n = 5), and CI-980 (n = 1). Three paclitaxel patients were missing surgery type. Abbreviation. KPS, Karnofsky performance status.

*Any bulk resection versus biopsy only.

Survival

Survival was assessed from the date of histologic diagnosis. Patients were observed until death or were censored as of October 2001, at which time the data were closed for this analysis.

Statistics

SAS software version 8.2¹⁷ (SAS Institute, Cary, NC) was used to perform survival analyses. Cumulative survival distributions were estimated using the Kaplan-Meier method. Confidence intervals (CI) for the failure rate were calculated using standard methods. This trial was designed and a sample size selected on the basis of a prospectively determined decision criterion that a phase III trial would be warranted if a more than 25% reduction in hazard rate for death was demonstrated in comparison with the outcome for patients treated on other NABTT experimental trials of agents shown to lack efficacy or be suboptimally dosed. These trials included patients treated with preirradiation paclitaxel, 9-AC, and CI-980. This reference population was initially thought to be appropriate because it included patients who elected to enroll onto experimental trials with similar eligibility criteria, and patients were treated at NABTT institutions during a similar time period. However, in retrospect, there were potential biases in using the NABTT trials as a comparison group. The trials involved delayed RT until after experimental chemotherapy. Because this potentially could have a negative effect on survival, the results of this comparison are reported, but a formal statistical analysis is not provided because it could be misleading.

RESULTS

Patients

Fifty patients with histologically confirmed newly diagnosed GBM were enrolled at five NABTT institutions from February 1998 through March 1999, whereas the trials that comprise the comparison group accrued patients from September 1993 through October 1999. Demographic and baseline clinical characteristics for patients treated on this protocol as well as the protocols included in the comparison group are listed in Table 1.

Toxicity

Thirty-eight (76%) of the 50 patients received \geq 27 (90%) of 30 planned RSR13 doses, and 42 (84%) of 50 patients received \geq 20 (67%) of 30 planned RSR13 doses.

Ten (20%) of the enrolled patients received less than 90% of the planned RSR13 doses because of adverse events and removal from therapy, including eight cases of RSR13-related dose-limiting toxicity and three unrelated events (sepsis, pneumonia, and pulmonary embolus). For the other two (4%) patients not completing at least 90% of the planned RSR13 doses, the reason was RSR13-related toxicity that allowed later resumption of dosing including hypoxemia¹ and renal dysfunction.¹ In all cases where patients failed to receive RSR13 during at least 67% of the RT treatments, the reason was adverse clinical events (8 of 10 RSR13 related) and required removal from the study. In total, 49 (98%) of 50 (98%) patients completed the planned 6-week RT course.

Twenty-four percent of patients experienced grade 3 or greater RSR13-related toxicity. These events are listed in Table 2. Except for one episode of fatal adult respiratory distress syndrome (ARDS), all toxicities were transient and self-limited. The patient who experienced fatal ARDS was receiving aromatic anticonvulsants and had additional findings suggestive of the anticonvulsant hypersensitivity syndrome, including rash, fever, flu-like symptoms, and histo-

Table 2. Drug-Related* Toxicity

Toxicity	No. of Patients		
	Grade 3	Grade 4	Grade 5
Nausea	1		
Headache	1		
Allergic rash	2		
ARDS-respiratory failure			1
Hypoxemia†	3		
Hypotension	1		
Creatinine increase, transient‡	1	1	
Sepsis, no neutropenia	1	1	

NOTE. Toxicity \geq grade 3 in 24%.

*Events at least possibly related to RSR13.

†Overnight admission for oxygen administration to maintain arterial oxygen saturation \geq 90%.

‡Three patients had grade 2.

logic evidence of peribronchial lymphadenopathy. A causative or contributory role for RSR13 in this fatal reaction could not be excluded.

A total of five patients had grade 2 or greater nonoliguric renal dysfunction, which was self-limited and resolved in all cases after interrupting RSR13 therapy. Two of the three patients who developed hypoxemia that required admission for continued supplemental oxygen administration were among the patients experiencing transient renal dysfunction. The cause of prolonged need for supplemental oxygen in the setting of renal dysfunction seemed to be reduced clearance of RSR13, resulting in prolonged PD effect. This is demonstrated by the case of the patient with grade 4 transient nonoliguric renal dysfunction who still had a RBC RSR13 concentration of 180 $\mu\text{g}/\text{mL}$ 4 days after receiving the drug.

PD Results

The mean \pm SD increase in p50 at the end of the RSR13 infusion was 11.96 ± 4.71 mmHg. This was not significantly different from the targeted shift of 10 mmHg. This represented a 43% increase from the mean baseline p50 of 27.44 mmHg. A total of 33 (66%) of 50 patients had a mean increase more than 10 mmHg, and 43 (86%) of 50 patients had a mean increase more than 8 mmHg. The PD effect was generally stable through the 6 weeks of dosing.

PK

The mean \pm SD end infusion plasma RSR13 concentration was 581 ± 75 $\mu\text{g}/\text{mL}$ (range, 437 to 828 $\mu\text{g}/\text{mL}$), and the end infusion RBC concentration was 560 ± 130 $\mu\text{g}/\text{mL}$ (range, 297 to 1063 $\mu\text{g}/\text{mL}$). The mean trough concentrations (ie, pre-RSR13 infusion) were 20 ± 29 $\mu\text{g}/\text{mL}$ (range, 0 to 170 $\mu\text{g}/\text{mL}$) and 14 ± 35 $\mu\text{g}/\text{mL}$ (range, 0 to 204 $\mu\text{g}/\text{mL}$). Mean concentrations of RBC and plasma concentrations remained generally stable for 6 weeks.

Relationship Between PD Effect and RSR13 Drug Concentration

PD effect (shift in p50; Dp50) was closely related to peak RBC concentration within this dosing range as follows: $\text{Dp50} = -0.49 \text{ mmHg} + [(0.0199 \text{ mmHg}/\mu\text{g}/\text{mL}) \times (\text{RSR13}_{\text{RBC}} \mu\text{g}/\text{mL})]$; $P = .0001$; $R^2 = 0.9$.

Survival

Forty-eight of the 50 patients accrued to this study have died. Follow-up time for the two surviving patients was more than 33 months. The total follow-up time of all patients was 55.49 years. Median survival was 12.3 months (95% CI, 9.3 to 13.8 months). The Kaplan-Meier survival curve is depicted in Fig 1. The failure rate, calculated as the number of deaths divided by person-years follow-up was

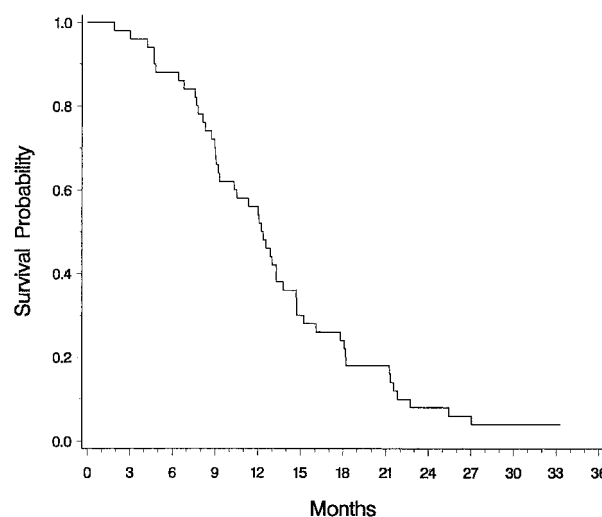


Fig 1. Kaplan-Meier survival curve for the 50 patients in the RSR13 phase II study. The two patients alive at the time of analysis were censored after 33 months.

0.87 (95% CI, 0.65 to 1.15). Six-month, 12-month, and 18-month survival rates are listed in Table 3.

In the trials within the NABTT CNS consortium that comprise the comparison group, 121 of 122 patients have died. The unadjusted hazard ratio comparing the death rate per patient-year for RSR13 to the reference group is 0.73 (95% CI, 0.52 to 1.03), and the hazard ratio adjusted for extent of surgery, Karnofsky performance status, and age is 0.72 (95% CI, 0.51 to 1.02). Median survival for the comparison population is 9.6 months (95% CI, 7.9 to 10.6 months), and 6-, 12-, and 18-month survival rates are listed in Table 3.

DISCUSSION

RSR13 is a synthetic allosteric modifier of hemoglobin that decreases the oxygen binding affinity of hemoglobin and augments oxygen unloading from the blood to hypoxic tissue. By increasing tissue oxygenation, RSR13 may reduce tumor hypoxia and enhance the cytotoxic effects of RT and chemotherapy. This study demonstrates that RSR13 at a dose of 100 mg/kg/d for 6 weeks during cranial RT for newly diagnosed GBM results in median survival in excess

Table 3. Survival Rates and 95% CI

Time	RSR13 Patients		Reference Patients	
	Survival Rate (%)	95% CI	Survival Rate (%)	95% CI
6 months	88.0	75.7-95.5	73.8	65.0-81.3
12 months	54.0	39.3-68.2	33.6	25.3-42.7
18 months	24.0	13.1-38.2	11.5	6.4-18.5

of 1 year, is a tolerable dosing regimen, and produces the expected PD effect on hemoglobin.

An agent such as RSR13, which increases tumor oxygenation, has the potential to improve the efficacy of RT. It has been known for several decades that hypoxic cells within tumors are profoundly radioresistant, requiring two and one half to three times as much radiation to achieve a similar cell kill as with normally oxygenated cells.¹⁵ The primary mechanism is thought to be the requirement for oxygen in the fixation of free radical mediated DNA damage, although hypoxia may also exert selective pressure for development of more malignant clones¹⁸⁻²⁰ and the cellular response to hypoxia may affect sensitivity to ionizing radiation.²¹⁻²⁵ Although the latter mechanisms may be less amenable to reversal by short-term delivery of oxygen, the former mechanism is likely to predominate because acute reversal of hypoxia has been demonstrated to result in immediate substantial increase in radiation response.²⁶⁻³⁰ In general, when the P_{O_2} reaches 10 to 20 mmHg, cells are fully radiosensitized.^{15,31} Therefore, only profoundly hypoxic tumor cells can be sensitized by increasing oxygen delivery, but already well-oxygenated normal tissues should not become more sensitive to RT, and normal tissue toxicity should not be worsened by enhanced oxygenation. Direct oxygen measurements in a variety of accessible human tumors have confirmed tumor hypoxia in carcinomas of the uterine cervix, head and neck, in breast carcinomas, and in GBM.^{5,32-41} Mean P_{O_2} of less than 10 mmHg have been shown to correlate with treatment failure even when controlling for other factors.³⁴⁻⁴¹

The dosing regimen of RSR13 used in this study was safe. The results demonstrate that most patients were able to receive this radiation-enhancing agent on a substantial portion of the RT treatment days. Seventy-six percent of patients received RSR13 on at least 27 of the 30 RT treatment days. Twenty-four percent of patients experienced \geq grade 3 toxicity that was transient and self-limited except in the case of an episode of fatal ARDS in a clinical setting consistent with anticonvulsant hypersensitivity, although the causative or contributory role for RSR13 in this fatality cannot be excluded. The potential role of an interaction of RSR13 with certain concurrently administered antihypertensive agents in the episodes of self-limited nonoliguric renal dysfunction is currently under investigation.

The observation of stable and predictable PK and PD effects of RSR13 confirmed the findings of the previous NABTT phase I study of RSR13. RSR13 dosing by body weight lead to relatively predictable drug concentrations that remained generally stable over 6 weeks. Similarly, a substantial and stable daily PD effect, as measured by the shift in p50, was reliably attained with this dosing regimen.

The selected PD goal was a 10-mmHg shift in p50, or approximately three times the normal physiologic response that occurs on high altitude acclimation. The mean peak daily shift attained was 11.96 ± 4.71 mmHg, a 43% shift from baseline, indicating that a significantly enhanced tendency toward oxygen unloading was achieved in this patient population. Notably, a substantial PD effect was achieved for most patients. Limited data from one patient treated on a NABTT trial who had brain tumor cyst fluid oxygen concentration measurements using an indwelling catheter demonstrated that the PD effect on hemoglobin observed with this dosing regimen can result in a substantial effect on oxygenation.⁴²

The median survival of patients treated with RSR13 seems favorable and exceeds the median survival observed in other NABTT trials. For patients treated with RSR13 in this NABTT trial, median survival was 12.3 months, and 1-year and 18-month survival rates were 54% and 24%, respectively. Although this study was designed with the prospective objective of comparing survival outcome for patients receiving RSR13 with the outcomes from other NABTT trials for newly diagnosed GBM, formal comparison is not presented here because the historical database is potentially flawed in a way that may create bias in favor of RSR13. The patients in the NABTT historical database received chemotherapy now known to have no efficacy in glioblastoma followed by delayed RT, which is an approach that could potentially have a negative impact on survival compared with immediate RT. The results of several phase II multi-institutional RTOG experimental trials, accruing patients from 1994 through 1997, were published in 2000 to 2001 and are available for comparison. These RTOG trial results included median survival of 9.7 months with concurrent paclitaxel and RT,⁴³ 9.1 months with carmustine (1,3-bis[2-chloroethyl]-1-nitrosourea; BCNU) and 64 Gy hyperfractionated RT,⁴⁴ 11.0 months with 70.4 Gy hyperfractionated RT and BCNU,⁴⁴ and 10.8 months and 9.5 months for patients treated with RT and two separate dosing levels of tirapazamine.⁴⁵ The preliminary results have been reported for an Eastern Cooperative Oncology Group randomized trial that accrued patients from 1996 to 1999.⁴⁶ Median survival was 11.2 months for concurrent BCNU and RT and was 10.7 months for neoadjuvant cisplatin and BCNU followed by delayed RT.

These data demonstrate that 100 mg/kg of RSR13 administered daily with cranial RT is tolerable and has an acceptable toxicity profile. RSR13 results in a substantial PD effect on hemoglobin for most patients treated with this dosing regimen. The median survival of patients treated with RSR13 is in excess of 1 year. A phase III randomized trial is under consideration. NABTT is now conducting a trial of RSR13 with BCNU in recurrent malignant glioma

and plans an imaging study to determine whether blood oxygen level-dependent MRI techniques can be used to measure RSR13-related tumor oxygenation changes.

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