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Michelle M Kim

Christina I Tsien

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Clinical Investigation

BRAINSTORM: A Multi-Institutional Phase 1/2 Study of RRx-001 in Combination With Whole Brain Radiation Therapy for Patients With Brain Metastases

Michelle M. Kim, MD,[*] Hemant A. Parmar, MD,[†] Matthew Schipper, PhD,[‡] Theresa Devasia, MS,[‡] Madhava P. Aryal, PhD,[*] Santosh Kesari, MD, PhD,[§] Steven O'Day, MD,[§] Aki Morikawa, MD, PhD,[‖] Daniel E. Spratt, MD,[*] Larry Junck, MD,[¶] Aaron Mammoser, MD,[#] James A. Hayman, MD,[*] Theodore S. Lawrence, MD, PhD,[*] Christina I. Tsien, MD,[**] Robert Aiken, MD,[††] Sharad Goyal, MD,[‡‡] Nacer Abrouk, PhD,[§§] Malcolm Trimble, MD,[‖‖] Yue Cao, PhD,[*] and Christopher D. Lao, MD[‖]

Department of Radiation Oncology, University of Michigan, Ann Arbor, Michigan; [†]Department of Radiology, University of Michigan, Ann Arbor, Michigan; [‡]Department of Biostatistics, University of Michigan, Ann Arbor, Michigan; [§]Providence Saint John's Health Center, John Wayne Cancer Institute, Santa Monica, California; [‖]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan; [¶]Department of Neurology, University of Michigan, Ann Arbor, Michigan; [#]Department of Neurosurgery, Louisiana State University, New Orleans, Louisiana; **Department of Radiation Oncology, Washington University School of Medicine, St. Louis, Missouri; [††]The Cancer Institute of New Jersey/Rutgers University, New Brunswick, New Jersey; [‡‡]Department of Radiation Oncology, George Washington University, Washington, DC; [§§]Clinical Trials Innovations, Mountain View, California; and [‖‖]Henry Ford Allegiance Health, Jackson, Michigan

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Purpose: To determine the recommended phase 2 dose of RRx-001, a radiosensitizer with vascular normalizing properties, when used with whole-brain radiation therapy (WBRT) for brain metastases and to assess whether quantitative changes in perfusion magnetic resonance imaging (MRI) after RRx-001 correlate with response.

Methods and Materials: Five centers participated in this phase 1/2 trial of RRx-001 given once pre-WBRT and then twice weekly during WBRT. Four dose levels were planned (5 mg/m^2, 8.4 mg/m^2, 16.5 mg/m^2, 27.5 mg/m^2). Dose escalation was

Corresponding author: Michelle M. Kim, MD; E-mail: michekim@med.umich.edu

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Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

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managed by the time-to-event continual reassessment method algorithm. Linear mixed models were used to correlate change in 24-hour T1, Ktrans (capillary permeability), and fractional plasma volume with change in tumor volume.

Results: Between 2015 and 2017, 31 patients were enrolled. Two patients dropped out before any therapy. Median age was 60 years (range, 30-76), and 12 were male. The most common tumor types were melanoma (59%) and non-small cell lung cancer (18%). No dose limiting toxicities were observed. The most common severe adverse event was grade 3 asthenia (6.9%, 2 of 29). The median intracranial response rate was 46% (95% confidence interval, 24-68) and median overall survival was 5.2 months (95% confidence interval, 4.5-9.4). No neurologic deaths occurred. Among 10 patients undergoing dynamic contrast-enhanced MRI, a reduction in Vp 24 hours after RRx-001 was associated with reduced tumor volume at 1 and 4 months ($P \leq .01$).

Conclusions: The addition of RRx-001 to WBRT is well tolerated with favorable intracranial response rates. Because activity was observed across all dose levels, the recommended phase 2 dose is 10 mg twice weekly. A reduction in fractional plasma volume on dynamic contrast-enhanced MRI 24 hours after RRx-001 suggests antiangiogenic activity associated with longer-term tumor response.

Introduction

Brain metastases are the most frequent intracranial neoplasms in adults and the most common neurologic complication of cancer. One-third to one-half of patients with brain metastases die of neurologic deterioration, and control of intracranial disease is correlated with increased survival.[1,2] However, systemic disease is most often the primary cause of death.

The standard of care for patients with 1 to 3 brain metastases otherwise eligible for local therapy is stereotactic radiosurgery (SRS) or, in select patients, surgery combined with SRS.[3-6] The addition of adjuvant whole-brain radiation therapy (WBRT) to local therapy reduces intracranial relapses and neurologic deaths,[2,7] and WBRT remains a standard of care for patients with >3-4 brain metastases and reasonable life expectancy. However, its efficacy may be limited in more radioresistant tumor types such as melanoma, in which uncontrolled intracranial disease and subsequent neurologic death remain an unaddressed issue.[8,9]

RRx-001 is a small molecule dinitroazetidine[10] whose potential radiosensitizing effects have been attributed to its effects on tumor vasculature and generation of nitric oxide (NO), an oxygen mimetic,[11] under hypoxic conditions endemic to tumors.[12] RRx-001 has also been shown preclinically to significantly increase tumor blood perfusion and to synergize with radiation therapy, likely secondary to enhanced oxygen delivery in the absence of neurotoxicity.[13] A phase 1 study in adult patients with advanced malignancies refractory to standard therapy demonstrated the feasibility and safety of RRx-001 as a single agent in doses of 10 mg/m^2 to 83 mg/m^2 with the absence of a maximally tolerated dose (MTD) or any dose-limiting toxicities (DLT).[14]

On the basis of its favorable toxicity profile and multiple preclinical studies demonstrating its potential activity as a radiosensitizer,[15] both systemically and in the brain, and as a normalizer of blood flow to gliomas,[16,17] the BRAINSTORM study was undertaken to determine the safety and efficacy of RRx-001 in combination with WBRT. Given the previously observed effects on tumor blood perfusion in systemically treated tumors, corollary dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) was performed to evaluate tumor-level effects on blood perfusion after treatment and whether quantitative changes after RRx-001 correlated with longer-term tumor response.

Methods and Materials

Study design

The trial was open to accrual at 5 medical centers: The University of Michigan (Ann Arbor, Michigan), Henry Ford Allegiance Health (Jackson, Michigan), Providence St. John's Health Center (Santa Monica, California), Washington University School of Medicine (St. Louis, Missouri), and The Cancer Institute of New Jersey/Rutgers University (New Brunswick, New Jersey). The trial was registered at ClinicalTrials.gov (NCT02215512). The institutional review boards of all participating institutions approved the study, which was conducted according to the provisions of the Declaration of Helsinki and the Good Clinical Practice Guidelines of the International Conference on Harmonization. Voluntary informed consent was obtained from all patients.

Eligibility criteria

This was a single-arm, open-label, multi-institutional phase 1/2 dose-escalation study in patients 18 years or older with a histologic diagnosis of solid tumor malignancy and radiographic evidence of 1 or more brain metastases on contrast-enhanced computed tomography (CT) or MRI of the brain. Additional eligibility criteria included an Eastern Cooperative Oncology Group

performance status of 0 to 2, life expectancy of at least 12 weeks, adequate hematologic, hepatic, and renal function as defined by hemoglobin ≥9 g/dL, absolute neutrophil count ≥1000 cells/mm^3, platelet count ≥50,000 cells/mm^3, creatinine ≤1.5 times the upper limit of normal (ULN) or a measured or calculated creatinine clearance of ≥50 mL/min, bilirubin ≤1.5 times ULN, alanine aminotransferase and aspartate aminotransferase ≤5 times ULN, and alkaline phosphatase <2.5 times ULN (≤5 times ULN with hepatic metastases).

Exclusion criteria included prior WBRT (prior radiosurgery was allowed); other systemic therapy within 2 weeks of first dose of study drug or required within 28 days from completion of treatment; prior RRx-001 therapy; unresolved toxicity higher than Common Terminology Criteria for Adverse Events (version 4.0) grade 1 attributed to prior therapy/procedure, excluding alopecia and hypothyroidism; serious comorbidities or findings on history, examination, or laboratory results that the investigator believed could interfere with study conduct or place the patient at unacceptable risk; inability to comply with study procedures or evaluations; and pregnancy or lactation. All patients were required to use acceptable methods of contraception for the duration of the study and for 28 days after withdrawal from study.

Treatment

Patients underwent standard CT simulation with immobilization in a thermoplastic mask for daily treatment. WBRT was 30 Gy in 3 Gy fractions delivered over 2 weeks using standard 6 MV photon opposed lateral fields.

RRx-001 was delivered on day −4 ± 2 days before the initiation of WBRT. It was then administered twice weekly on days 1, 4, 8, and 11 (±1 day) during the course of WBRT and before radiation treatment on infusion days (Fig. 1). For the first 9 patients, RRx-001 was given directly intravenously, but owing to localized discomfort and vasodilation on infusion due to localized NO release, the study was amended to change the method of administration. All subsequent patients received ex vivo mixed anticoagulated autologous blood (12 mL), which induced the NO release before administration. The RRx-001−autologous blood mixture was then given intravenously within 1 hour from the time of blood collection to minimize risk of contamination (Appendix E1; available online at https://doi.org/10.1016/j.ijrobp.2020.02.639).

After preliminary data demonstrated potential synergy between RRx-001 and temozolomide, a subsequent study amendment allowed concurrent (75 mg/m^2 daily during WBRT) and adjuvant (200 mg/m^2 on days 1-5 of a 28-day cycle) delivery of temozolomide with RRx-001. Seven patients were treated on this amendment.

Dose escalation

The primary objective was to determine the MTD associated with a 20% probability of DLT. Four dose levels were planned for twice weekly administration (5 mg/m^2, 8.4 mg/m^2, 16.5 mg/m^2, 27.5 mg/m^2), which was amended to a similar flat twice weekly dosing scheme (10 mg, 17 mg, 33 mg, 55 mg) when the method of intravenous administration changed. The first patient was treated at RRx-001 dose level 1. Each subsequent patient's dose level was assigned using the time-to-event continual reassessment method algorithm.[18,19] When a new patient was enrolled, the probability of toxicity at each dose was estimated, based on the toxicity data accrued up to that time, using a 1-parameter logistic dose-toxicity model. The patient was assigned to the highest dose with estimated probability of toxicity closest to the target rate of 20% but not exceeding 25%, subject to 2 dose-escalation restrictions: (1) no previously untried dose levels could be skipped, and (2) at least 2 patients must have completed the prior dose level before a patient could be treated at the next dose level. Dose escalation was resumed from dose level 1 after the study amendment using the modified method of intravenous administration.

Safety assessments

All patients who received at least 1 dose of study treatment, defined as RRx-001 and/or WBRT, comprised the safety analysis population. All treatment-emergent adverse events

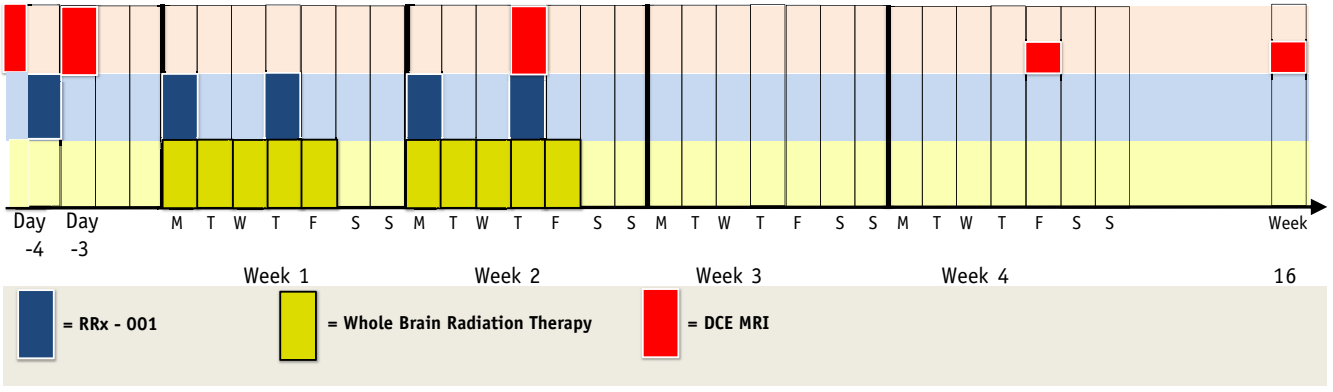

Fig. 1. Study design.

(AEs) were evaluated, coded, and assigned a grade using the National Cancer Institute Common Toxicity Criteria version 4.03. An AE was considered treatment emergent if it increased in severity over the baseline severity grade and if the date of onset occurred after enrollment but no more than 28 days after the last dose of study treatment. DLT was defined as any grade 3 or higher toxicity possibly, probably, or definitely related to RRx-001 treatment. The attribution and relationship of the event to RRx-001 was assessed by the investigator. AEs attributed to WBRT alone were not considered DLTs.

Efficacy assessments

Secondary objectives included best intracranial overall response rate (ORR, complete response [CR] and partial response [PR]) and overall survival (OS). Response was assessed using modified Response Evaluation Criteria in Solid Tumors 1.1 because the study was initiated before the availability of the Response Assessment in Neuro-Oncology Brain Metastases Criteria[20] were available. A CR was defined as complete resolution of all enhancing target lesions. A PR was defined as at least a 30% reduction in the sum of the longest diameters of enhancing target lesions compared with baseline sum of longest diameters and an absolute decrease of at least 5 mm in at least 1 target lesion. Progressive disease was defined as an increase of $\geq$20% in the sum of the longest diameters of target lesions, taking as reference the smallest sum on study and an absolute increase in size of at least 5 mm in at least 1 target lesion, or the appearance of 1 or more new lesions at least 6 mm in size. All other evaluations were reported as stable disease (SD). Lesions that had received prior radiosurgery or were nonenhancing and primarily hemorrhagic were not considered evaluable for RECIST response.

There were 22 patients treated with WBRT + RRx-001 alone with sufficient efficacy data (OS and ORR), and the Kaplan-Meier method was used to derive an estimate of the median and its corresponding 95% confidence interval (CI). ORR was estimated by counting the number of patients experiencing a CR or PR. Patients with at least 1 documented response assessment were included in the analysis of antitumor activity.

Statistical analyses were performed using R Core Team (2013). Statistical inferential tests benchmarking uses a .05 2-sided significance level. CI estimates were 2 sided and set at 95%.

Advanced MRI correlation

Patients enrolled at The University of Michigan underwent correlative DCE MRI at baseline (within 21 days before the first dose of RRx-001), within 24 hours after the first dose of RRx-001 but before WBRT, at the end of WBRT but before the last fraction of RT, and 1 month and 4 months after completion of WBRT. Patients remained off standard-of-care systemic therapies for all imaging time points from at least 2 weeks before the baseline scan, up to and including 1 month post-WBRT, to eliminate confounding effects of other nonprotocol therapies.

MRI acquisition and image processing

All MRI scans were performed on a 3T scanner (Skyra, Siemens Healthineers, Erlangen, Germany) using a 20-channel head coil in the Department of Radiation Oncology at The University of Michigan. Conventional 3-dimensional pre- and postcontrast T1-weighted images and 2-dimensional T2- fluid-attenuated inversion recovery images were acquired.

DCE images were acquired by a 3-dimensional gradient echo pulse sequence, called TWIST, in the sagittal orientation to avoid in-flow effect and ensure arterial coverage for input function delineation. The details of image acquisition and processing have been previously published in an unrelated advanced imaging study using the same method.[21] Briefly, a 3-parameter Tofts model was used to quantify DCE MRI to obtain the fractional plasma volume (V_p), transfer constant of contrast (K^{trans}), and fractional volume of extravascular extracellular space (v_e)[22] and was implemented using an in-house functional image analysis tool (*im*FIAT).[23-26] V_p is directly related to cerebral blood volume (a density measurement) by unit conversion, and V_p, cerebral blood volume, and cerebral blood flow are highly correlated in brain tumors.[27] A full characterization of the performance of software using digital reference objects with a large range of physiologic parameters, acquisition parameters, and added Gaussian noise has been previously published.[23]

Image review and volume delineation

MRI scans at all acquired time points were centrally reviewed with the study neuroradiologist. Lesions were delineated on the T1-weighted postgadolinium image at each time point. The mean T1, V_p, K^{trans}, and V_e in each lesion at each time point were calculated.

Statistical analysis of imaging study

Mixed effect regression models were used to assess the relation between baseline or during-treatment imaging features and later changes in tumor volume. Subject-specific random effects were included to account for possible correlation between multiple tumors within a patient. A stepwise model-building approach with a P value threshold of .05 was used to build a multivariable model in which all included terms were jointly significant. Potential covariates included RRx-001 dose level and baseline 24-hour post-RRx change (compared with baseline) and end of WBRT + RRx change (compared with baseline) in quantitative T1, K^{trans}, and V_p.

Results

Study population

Between February 2015 and February 2017, 31 patients were enrolled on study. Two patients dropped out at baseline before receiving study therapy because of rapid clinical deterioration, and 7 patients were treated on a separate amendment with concurrent RRx-001 and temozolomide; their tumor outcomes will be reported in a separate publication.

Median age among the 22 evaluable patients was 60 years (standard deviation 13 years), and 12 (55%) were male. The most common primary tumor histologies were melanoma (59%) and non-small cell lung cancer (18%). Demographic and baseline characteristics are summarized in Table 1. Patients with a limited number of lesions underwent WBRT on protocol per physician discretion, usually in the setting of uncontrolled systemic disease. Melanoma was the most common tumor histology because of referral patterns and the specialization of referring physicians.

Table 1 Demographics and baseline characteristics

Characteristic	No. of patients (%)
Age, mean 59.2 y (SD = 12.9, median 59.6)	22
≤60 y	13 (59.1)
>60 y	9 (40.9)
Sex	
Male	12 (54.5)
Female	10 (45.5)
Primary tumor type	
Melanoma	13 (59.1)
NSCLC	4 (18.2)
Breast	2 (9.1)
SCLC	1 (4.5)
Gastrointestinal	1 (4.5)
Bladder	1 (4.5)
ECOG performance status	
ECOG 0	7 (31.8)
ECOG 1	13 (59.1)
ECOG 2	2 (9.1)
Missing	0 (0.0)
No. of brain metastases	
1	0 (0.0)
2-3	1 (4.5)
≥4	19 (86.4)
Missing	2 (9.1)
Extracerebral metastases	
Yes	21 (95.5)
No	1 (4.5)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer; SD = standard deviation.

Toxicity

No DLTs were observed at any dose level. Five patients were treated at dose level 3, which was the highest dose level achieved owing to a study amendment based on preclinical data showing efficacy at lower dose levels before dose level 4 was reached (Table 2).

Among 29 patients who received at least 1 dose of RRx-001, the majority of AEs at least possibly related to study treatment were grade 1 (mild) to grade 2 (moderate) in severity. The only grade 3 treatment-emergent AE ≥5% was asthenia in 2 of 29 patients (6.9%), neither of which were attributed to RRx-001. There were no grade 5 adverse events. The most common grade 1 or 2 AEs ≥5% are shown in Table E1 (available online at https://doi.org/10.1016/j.ijrobp.2020.02.639). All grade 3 or 4 AEs are shown in Table E2 (available online at https://doi.org/10.1016/j.ijrobp.2020.02.639). The only AEs of any grade ≥5% attributed to the combination of RRx-001 and WBRT were grade 1 to 2 infusion-related reaction in 9 of 29 patients (31%), grade 1 to 2 fatigue in 2 of 29 patients (6.9%), and grade 1 to 2 headache in 2 of 29 patients (6.9%). There were no grade 3, 4, or 5 treatment-related AEs.

Intracranial response assessment

In the 22 patients who completed treatment and received only RRx-001 and WBRT, best intracranial response rates to the treatment were as follows: 1 CR, 9 PRs, 5 SD, 1 progressive disease, and 6 nonevaluable patients (Table 3). Among 22 evaluable patients, 13 (59%) had melanoma. Intracranial disease control rates were 70% for patients with melanoma (6 PR and 3 SD) and 66% for non-melanoma tumor types (1 CR, 3 PR, and 2 SD).

Survival

Mean and median duration on study for the 15 patients experiencing a response (CR or PR) or SD were approximately 11.3 and 5.7 months, respectively (Fig. 2).

The median survival duration for 22 evaluable patients was 5.2 months (95% CI, 4.5-9.4). The corresponding OS Kaplan-Meier curve is depicted in Figure 3. Intention-to-treat (n = 31) median survival was 5.4 months (95% CI,

Table 2 Estimated rates of dose-limiting toxicity per dose level

Dose level	Posterior estimate	No. DLTs	No. treated	Prior estimate	Lower 95% CI	Upper 95% CI
1	0.009	0	8	0.05	0.001	0.070
2	0.018	0	9	0.08	0.002	0.108
3	0.027	0	5	0.11	0.003	0.144
4	0.043	0	0	0.15	0.006	0.190

Abbreviations: CI = confidence interval; DLT = dose limiting toxicities.

Table 3 Intracranial response summary

Response*	Count	Evaluable percentage	ITT± percentage
Complete response	1	5	3
Partial response	9	41	29
Stable disease	5	23	16
Progressive disease	1	5	3
Nonevaluable	6	27	19
Overall response†	10	46	32
Disease control‡	15	68	48

Abbreviation: ±ITT = intention to treat population.

* Response was assessed at 1 and 4 months using modified RECIST 1.1. Complete response: complete resolution of all enhancing lesions. Partial response: ≥30% reduction in sum of longest diameters of enhancing target lesions vs baseline sum of longest diameters and absolute decrease of ≥5 mm in at least 1 target lesion. Progressive disease: ≥20% increase in sum of longest diameters of target lesions vs smallest sum and absolute increase of ≥5 mm in at least 1 target lesion, or ≥1 new lesion at least 6 mm. All other evaluations reported as stable disease.

† Overall response = complete response + partial response

‡ Disease control = complete response + partial response + stable disease

4.5-12.4). All patients but 1 had active extracranial disease on initial presentation. No patients died of progression of intracranial disease.

Correlative DCE-MRI

Twelve patients underwent correlative DCE-MRI and 2 patients were excluded from imaging analysis because of receipt of prior SRS to all lesions and rapid clinical deterioration with insufficient follow-up, respectively. Ten patients underwent DCE MRI scans with 64 total lesions evaluable at baseline, 24 hours, and end of RT. Eight patients with 44 total evaluable lesions had available imaging at 1 month, and 6 patients with 29 total evaluable lesions had imaging at 4 months. Baseline characteristics of the 10 evaluable patients undergoing DCE MRI are listed in Table 4.

On univariate analysis, only a decrease in 24-hour V_p from baseline after a single dose of RRx-001 was marginally associated with absolute tumor volume response 1 month after treatment ($P = 0.07$, Table E3; available online at https://doi.org/10.1016/j.ijrobp.2020.02.639).

In a stepwise multivariate model assessing factors associated with tumor volume response, RRx-001 dose

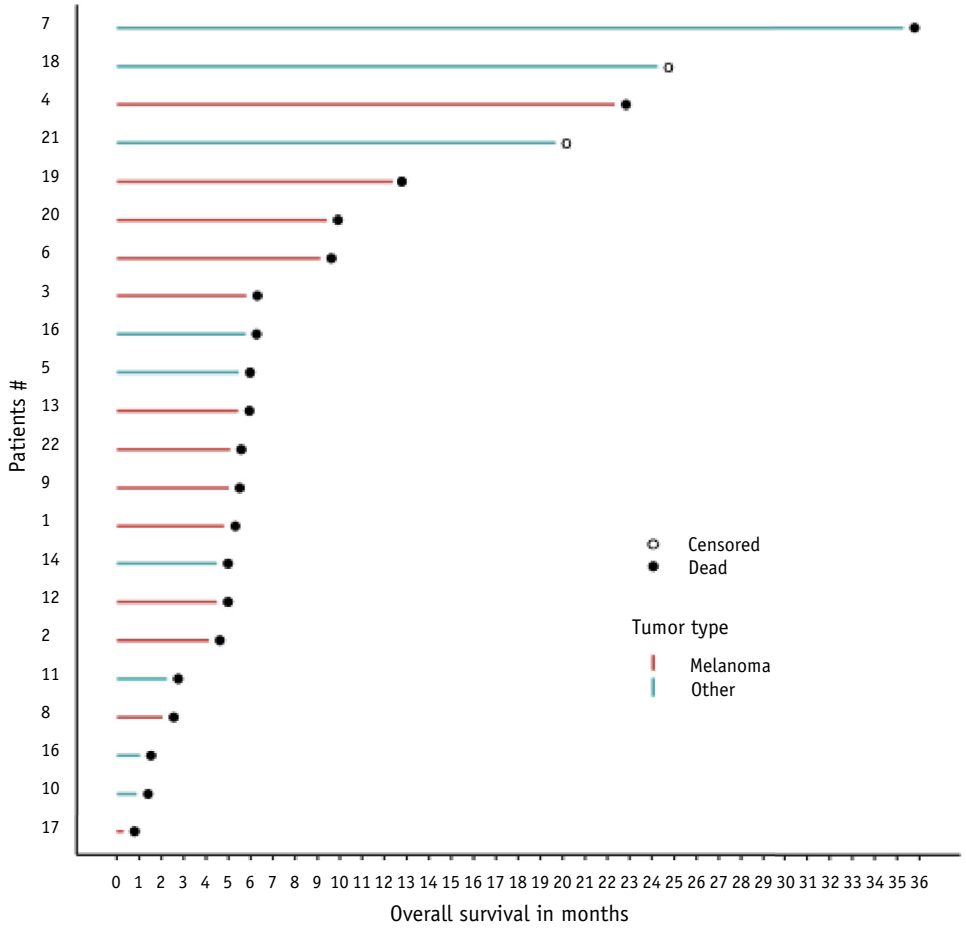

Fig. 2. Durability of response and survival by tumor type (N = 22).

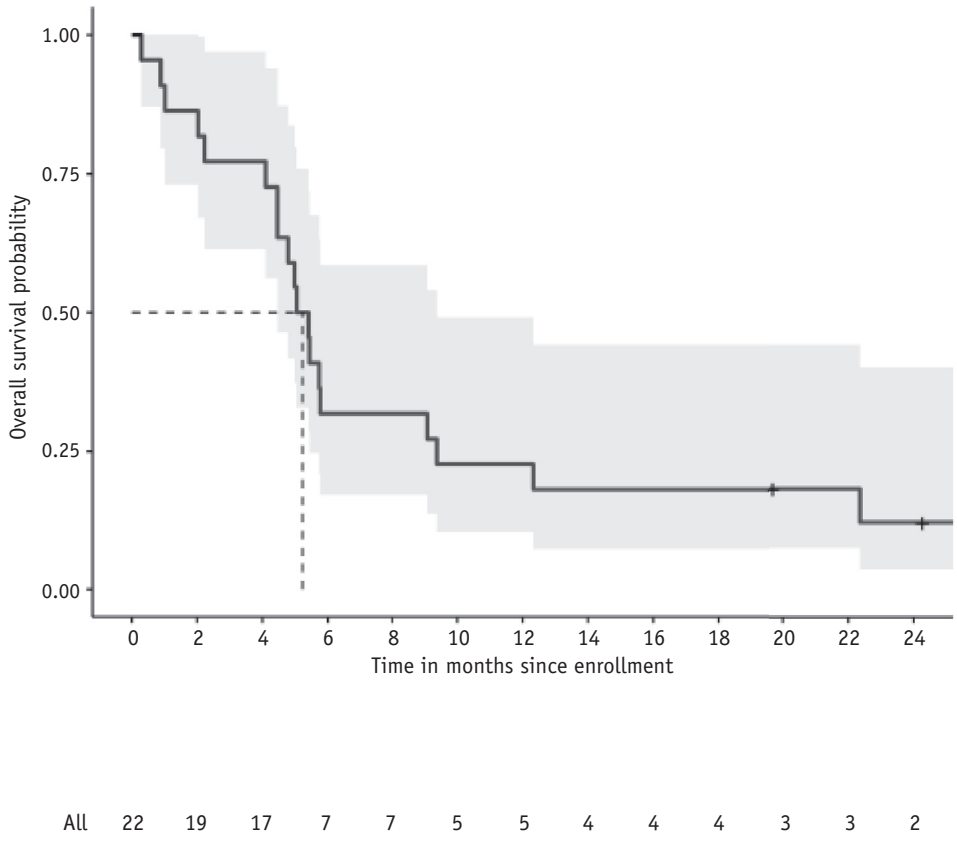

Fig. 3. Overall survival Kaplan-Meier curve (with 95% confidence interval indicated in shading; N = 22).

level and baseline 24-hour post-RRx change (compared with baseline) and end of WBRT + RRx change (compared with baseline) in quantitative T1, K^{trans}, and V_p were entered. Only V_p before therapy and 24-hour change in V_p remained significant and were retained in the model after stepwise selection. A reduction in V_p 24 hours after RRx-001 (before WBRT) was associated with reduced tumor volume at 1 month (estimate 0.88; 95% CI, 0.37-1.40; P = .001) and 4 months (estimate 1.51; 95% CI, 0.58-2.43; P = .003). Likewise, a lower V_p before therapy was associated with reduced tumor volume at 1 month (estimate 0.73; 95% CI, 0.29-1.17; P = .002) and 4 months (estimate 1.8; 95% CI, 0.95-2.65; P = .0002), suggesting antiangiogenic activity and early potential vascular normalization after a single dose of RRx-001 as predictive of longer-term tumor response.

Table 4 Baseline tumor, treatment, and imaging characteristics of evaluable patients undergoing DCE-MRI (N = 10)

Patient	Primary tumor type	Subtype	Sex	Baseline steroids	RRx dose level	No. of lesions*	Median lesion volume (range), cm^3	Systemic therapy after WBRT + RRx
1	Melanoma	BRAFwt	Male	Yes	1	5	1.9 (0.2-10.8)	ICI
2	Melanoma	BRAFm	Male	No	1	3	0.3 (0.2-0.4)	ICI
3	Melanoma	BRAFm	Male	No	1	4	0.8 (0.3-2.7)	BRAFi, ICI
4	Melanoma	BRAFm	Female	No	1	3	0.4 (0.3-1.5)	ICI, TMZ
5	NSCLC	UNK	Female	No	2	13	0.1 (0.04-0.5)	ICI
6	Breast	ER+/PR+/HER2+	Female	No	2	4	0.4 (0.1-1.0)	Trastuzumab, pertuzumab
7	Melanoma	BRAFwt	Female	Yes	3	13	0.8 (0.1-10.1)	None
8	Melanoma	BRAFwt	Female	No	3	4	0.3 (0.2-0.3)	ICI
9	NSCLC	UNK	Female	Yes	3	7	0.1 (0.09-0.4)	None
10	Melanoma	BRAFwt	Male	No	3	8	0.4 (0.2-0.8)	TMZ

Abbreviations: BRAFi = BRAF inhibitor; ICI = immune checkpoint inhibitor; NSCLC = non-small cell lung cancer; TMZ = temozolomide.
* Lesions evaluable at baseline (not previously irradiated); units 1/ms; units 1/min; units mL/g.

Discussion

BRAINSTORM is the first trial to evaluate the safety and tolerability of concurrent RRx-001 with WBRT for patients with brain metastases. The combination was well tolerated with no safety signals at all doses tested. No MTD was reached, and no DLTs were observed. The majority of AEs were mild to moderate in severity, and none resulted in clinical trial discontinuation. Additionally, the novel finding that early tumor blood volume reduction 24 hours after RRx-001 was associated with longer-term tumor response suggests early potential antiangiogenic effects of RRx-001 at the level of tumor vasculature that may serve as a potential biomarker of early treatment response, warranting further investigation in future studies.

Potential synergy between RRx-001 and WBRT is suggested by an intracranial response rate of 46% among evaluable patients (32% among the ITT population), which is notable because the majority of patients treated on this study had melanoma, a typically radioresistant tumor type in which response rates after WBRT have been reported to be as low as 5%.[28] Intracranial disease control rates among patients with melanoma (70%) were as high as those in patients with more radiosensitive tumor types (66%). However, response rates beyond 1 month in this study may have been confounded by the effect of subsequent systemic therapies with potential intracranial activity such as immune checkpoint inhibitors, which many patients received. Overall survival was 5.2 months in this poor-prognosis cohort with active extracranial disease, but it is notable that no patients died of neurologic causes despite many patients having a significant burden of presumably radioresistant intracranial disease.

The mechanism for the potential synergy between RRx-001 and radiation is hypothesized, at least in part, to be related to nitric oxide generation under hypoxic tumor conditions and normalization of the tumor vasculature, which may selectively enhance the effects of radiation therapy at the tumor level.[15] Tumor hypoxia as a source of radioresistance has been well documented in preclinical and clinical studies over many decades, with evidence of improved locoregional tumor control with abrogation of tumor hypoxia in certain tumor sites such as the head and neck.[29] Studies have demonstrated that upon administration, RRx-001 penetrates the red blood cell (RBC) membrane and irreversibly binds to the beta Cysteine 93 residue of hemoglobin.[30] Subsequently induced RBC membrane changes lead to increased phosphatidylserine exposure, and RRx-001−modified RBCs are preferentially internalized and catabolized by the endothelial cells of the microvasculature, releasing redox active metabolites and causing oxidative damage.[31] Studies have previously demonstrated that RRx-001 exerts a cytotoxic effect in vitro against a variety of human cancer cell lines, an effect that is enhanced under hypoxic conditions.[10,13] In HT29 and SCC VII cell lines, RRx-001 induces intracellular reactive oxygen species generation, leading to DNA damage and tumor cell apoptosis.[13] Inhibition of tumor growth was also observed in vivo in mice bearing SCC VII tumors and demonstrated selective tumor cell radiosensitization in vitro and in vivo under hypoxic and normoxic tumor conditions, with prolongation of tumor growth delay times for combination therapy without a reduction in animal body weight compared with untreated control or radiation-treated mice.[13]

Our observation of tumor-level vascular effects offers a first-time look at the potential effect of RRx-001 for metastatic tumors in the central nervous system. Prior preclinical studies[13] using microbubble-enhanced ultrasound imaging to assess flank tumors in mouse models before and after RRx-001 and radiation demonstrated a dramatic increase in the rate of blood perfusion and blood volume of SCC VII tumors in a dose- and time-dependent manner that peaked 6 hours after administration of RRx-001. In this study, we performed a lesion-level analysis of quantitative metrics derived from DCE MRI in patients with intracranial metastases from solid tumor malignancies. We demonstrated a significant and consistent relationship between reduced blood plasma volume (V_p) 24 hours after administration of RRx-001 alone and subsequent tumor volume response; this is the first advanced imaging metric linked to clinical response in patients with brain metastases treated with RRx-001. A number of explanations are possible for the observation of reduced V_p 24 hours after administration of RRx-001 in our study, including the assessment of metastatic brain tumors in human patients, who may exhibit different phenotypic features at the level of brain tumor vasculature compared with the flank tumors in mouse subjects previously studied. Moreover, the use of quantitative DCE-MRI to assess fractional plasma volume and cerebral blood volume changes after RRx-001, as well as the timeline (24 hours) selected for assessment in our study, stands in contrast to the methodology used in prior preclinical studies, in which ultrasound imaging of systemic disease was acquired as early as 2 to 6 hours after RRx-001 administration.

A possible explanation for this presumed early (24 hour) effect of RRx-001 on metastatic brain tumor response may be related to a direct antiangiogenic effect induced through RRx-001 catabolism and oxidative damage. Metastatic brain tumors, like other solid tumor malignancies, depend on vascular blood supply for outgrowth. Mechanisms of achieving vascular nutrient supply appear to vary by tumor histology, with melanoma brain metastases demonstrating a tendency for a co-optive growth pattern along pre-existing vascular structures.[32,33] Whether RRx-001 induces antiangiogenic effects against pre-existing vascular channels in this cohort of patients with predominantly melanoma brain metastases was not directly evaluated in this study, but the reduction in blood plasma volume associated with tumor response suggests tumor vascular effects by RRx-001 in the central nervous system that merit further investigation in future planned studies.

Conclusions

RRx-001 in combination with WBRT appears well tolerated in patients with brain metastases, with an intracranial response rate of 46%, an intracranial disease control rate of 68%, and no neurologic deaths observed. After a single dose of RRx-001, a reduction in V_p assessed by DCE MRI is associated with tumor volume response at 1 month and 4 months and may potentially serve as a biomarker of longer-term response. Because activity was observed across all dose levels in the absence of an observed dose-response, the recommended phase 2 dose is 10 mg administered twice weekly.

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