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Results of a phase I trial to assess the safety of macitentan in combination with temozolomide for the treatment of recurrent glioblastoma

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Abstract

Background. There is an urgent need for additional therapies to treat recurrent glioblastoma (GBM). Preclinical studies suggest that high dose macitentan, an oral dual endothelin receptor antagonist, enhances the cytotoxic effects of temozolomide (TMZ) in GBM, improving survival. This phase I trial investigated the maximum tolerated dose of macitentan combined with TMZ in patients with recurrent GBM and assessed the safety and tolerability of high dose macitentan in these patients (NCT01499251).

Methods. Adults with recurrent GBM received ascending doses of macitentan from 30 mg once daily concomitantly with TMZ. Safety and tolerability were assessed in addition to exploratory efficacy and pharmacokinetic endpoints. An ancillary study examined biomarker expression following macitentan treatment prior to surgical resection of recurrent GBM.

Results. Thirty-eight patients with recurrent GBM were administered macitentan doses up to 300 mg once daily; no dose-limiting toxicities were observed, and a maximum tolerated dose was not determined. All patients experienced at least one treatment-emergent adverse event (TEAE), the majority associated with GBM or TMZ treatment. TEAEs related to macitentan and TMZ were reported for 16 (42.1%) and 26 (68.4%) patients, respectively, with no serious macitentan-related TEAEs. Macitentan concentrations increased with dose, with no plateau in exposure. Substantial heterogeneity was observed in the expression of efficacy biomarkers within tumors. The Kaplan-Meier estimate of median overall survival across all dose groups was 9.4 (95% CI 8.5, 13.4) months.

Conclusion. High-dose macitentan was well tolerated in recurrent GBM patients concomitantly receiving TMZ. TEAEs were consistent with those seen in patients receiving either drug individually.

Key Points

- High-dose macitentan was well tolerated in recurrent glioblastoma patients.
- No mutual PK interaction between macitentan and temozolomide was evident.
- Expression of efficacy biomarkers within individual tumors was heterogeneous.

Glioblastoma (GBM) is the most common and aggressive primary brain tumor.[1] For patients newly diagnosed with GBM, the standard of care includes maximal safe surgical resection followed by radiotherapy plus concomitant temozolomide (TMZ) and adjuvant therapy with cyclic TMZ.[2] In more than 90% of patients, GBM inevitably recurs,[2,3] and management

Importance of the Study

Glioblastoma is the most common malignant primary brain tumor in adults and invariably carries a poor prognosis. Recurrence is inevitable and salvage therapies are limited underscoring this unmet therapeutic need. Glioblastomas produce endothelins, and the expression of their receptors (endothelin A [ET-A] and B [ET-B] receptors) is increased in glioblastoma. Stimulation of these receptors influences neovascularization and tumor progression. Macitentan, a dual endothelin receptor antagonist approved for the treatment of pulmonary arterial hypertension, has demonstrated efficacy in preclinical tumor models and has a good safety profile. A phase I trial examined macitentan combined with dose-dense temozolomide in recurrent glioblastoma patients. Results demonstrated that macitentan is well tolerated at doses far higher than those used for the treatment of pulmonary hypertension.

can involve further surgical resection, chemotherapy, re-irradiation, bevacizumab (Avastin®) or, in a minority of patients, investigational therapies.[2]

Bevacizumab, a humanized monoclonal antibody targeting vascular endothelial growth factor, was approved in 2009 in the USA for recurrent GBM.[4,5] Although bevacizumab use has been shown to improve progression-free survival (PFS), this has not led to a meaningful overall survival (OS) benefit.[4] Lomustine, an alkylating cytotoxic chemotherapy, is often used as a salvage therapy in recurrent GBM,[6] primarily because other treatments are yet to demonstrate superior results in randomized controlled trials.[4] However, PFS following lomustine at 6 months remains low (15–25%).[4] Other approaches for the treatment of recurrent GBM include dose-dense TMZ (ddTMZ) regimens, which have shown higher PFS at 6 months than standard TMZ dosing.[7] Irrespective of the treatment strategy chosen, prognosis remains poor,[2] with a median survival of 14.6 months following the initial diagnosis of GBM.[3] As such, there remains a significant unmet medical need for additional treatments, and a number of therapies targeting novel pathways involved in the GBM pathology are currently under investigation.[1]

The endothelin axis is recognized to promote growth and differentiation in a number of cancers, and the expression of endothelin A (ET-A) and B (ET-B) receptors is markedly increased in GBM.[8,9] Stimulation of these receptors, located on both tumor and endothelial cell membranes, ultimately promotes tumor progression and neovascularization.[10] Macitentan is an oral, dual endothelin receptor antagonist (ERA), approved at a 10 mg once-daily dose for the treatment of pulmonary arterial hypertension (PAH).[11] In preclinical *in vitro* experiments, dual endothelin receptor antagonism with macitentan reversed glioma cell TMZ chemoprotection, afforded by astrocytes and endothelial cells.[10] Furthermore, a murine orthotopic model of glioblastoma showed that macitentan in combination with ddTMZ downregulated markers of cell survival (pAKT and MAPK) whilst greatly enhancing apoptosis in both glioma and tumor-associated endothelial cells. A second orthotopic murine model, established using a TMZ-resistant LN-229 cell line, demonstrated that the combination of macitentan with ddTMZ dramatically prolonged survival.[12] In contrast, targeting only the ET-A receptor using zibotentan did not result in any increase in survival in the orthotopic murine model, compared to ddTMZ alone.[12]

In addition to the preclinical models of GBM described above,[12] an additional study also examined the impact of combined macitentan and paclitaxel in a murine model of ovarian cancer, reporting inhibition of survival pathways and reduced tumor progression.[13]

Importantly, these preclinical studies have demonstrated macitentan efficacy using doses (per kg) substantially higher than the once-daily dose of 10 mg approved for use in PAH.[12] Toxicity studies performed in rats and dogs have indicated that, at an exposure level equivalent to 30 mg/day in humans, macitentan administration was safe (unpublished data). Preclinical data are supported by safety studies in healthy human volunteers, in which macitentan doses of up to 300 mg/day were well tolerated.[14,15] Together, these studies provided a strong preclinical and clinical rationale for human studies combining high dose macitentan with ddTMZ in GBM.

This manuscript reports the results of a prospective, single-center, open-label, phase I multiple ascending dose study used to examine the safety and tolerability of macitentan in patients with recurrent GBM. The study aimed to expand safety information on the maximum tolerated dose (MTD) of macitentan with ddTMZ in patients with recurrent GBM. Exploratory efficacy endpoints were assessed in addition to treatment-emergent adverse events (TEAEs) of special interest based on the safety profile of macitentan observed in other indications. An ancillary study aimed to determine changes in the expression of biomarkers associated with target modulation and pharmacodynamics.

Materials and Methods

Study Population

This study enrolled adults with histologically confirmed WHO grade IV GBM at first recurrence who were at least 3 months from completion of concurrent chemoradiation. Patients were required to have a Karnofsky Performance Status (KPS) ≥ 60 with adequate bone marrow function and to have previously tolerated TMZ without the need for transfusion. The study was conducted in accordance with the Declaration of Helsinki and a local institutional

review board approved the protocol. Informed consent was obtained from patients before study entry and any mandated study procedure. The study was monitored throughout by a safety monitoring committee (SMC), including assessment of dose-limiting toxicities (DLTs).

Study Design and Treatment

The study (NCT01499251) assessed the safety and tolerability of ascending doses of oral macitentan given concomitantly with oral ddTMZ. Samples were collected from patients enrolled for treatment at the MD Anderson Cancer Center, Houston Texas.

Macitentan was administered once-daily 7-days prior to ddTMZ (150 mg/m^2) and was given throughout all treatment periods. Modification of ddTMZ dosing was permitted for new cohorts following review by the SMC. Given that, at the time of study initiation, there was no standard of care for recurrent GBM patients, that a similar ddTMZ schedule (150 mg TMZ for 7-days every two weeks) had reported significant reductions in O^6-Methylguanine-DNA-methyltransferase (MGMT) activity,[16] and coupled to the positive preclinical data where combination of ddTMZ and macitentan were found to be efficacious,[12] a ddTMZ regimen was utilized in this study.

Once ddTMZ treatment was initiated, patients alternated treatment periods of 7 days on and 7 days off for up to 12 cycles, each cycle lasting 28 days (Supplementary methods). DLTs were assessed within the first cycle of macitentan plus ddTMZ. End of treatment (EOT) occurred when a patient discontinued macitentan and ddTMZ after completing 12 cycles of treatment or when study treatment was prematurely discontinued for any reason. The study was comprised of a phase I and phase Ib component with an ancillary biomarker study (Figure 1).

Phase I Study

A conventional 3+3 dose escalation design[17] was used to determine the MTD of macitentan in combination with ddTMZ. If 0/3 patients experienced a DLT and there was no plateau in macitentan plasma levels, 3 additional patients were enrolled and treated at the next higher dose level. The starting dose of macitentan was 30 mg once-daily which increased in 30 mg increments. The MTD was to be defined as the highest dose of macitentan at which 0/3 or < 2/6 patients experienced a DLT (Supplementary methods).

Phase Ib Study

The phase Ib study was designed to expand on the safety and tolerability data, using the recommended macitentan and ddTMZ dosing schedule determined from the phase I dose-escalation study. The SMC selected the 150 mg once-daily macitentan dose for evaluation in the phase Ib cohort (Figure 1). Please refer to the Supplementary methods and ET-1 plasma results (Supplementary Figure 1) for details on the rationale for selecting this dose of macitentan in the phase Ib study.

Ancillary Study

An ancillary study was performed to evaluate the effects of macitentan with ddTMZ on brain tumor tissue biomarkers and macitentan safety and tolerability in patients with recurrent GBM undergoing craniotomy. Patients were treated with macitentan at an assigned dose selected by the SMC (150 mg or 120 mg), starting 8–14 days before planned surgery. Brain tumor tissue was resected during surgery for biomarker assessment. Macitentan treatment was stopped 1 day before surgery and then restarted at the same dose after recovery, 3–5 weeks after surgery. Where available, biomarker staining was performed on samples of the primary tumor, and brain tumor tissue resected following recurrence. This provided an opportunity to compare biomarker staining intensity pre- and post-macitentan treatment.

Study Endpoints and Outcome Measures

The primary objective of the study was to determine the MTD of macitentan in combination with ddTMZ. Safety and tolerability assessments included measurement of TEAEs, serious adverse events (SAEs), TEAEs leading to study treatment discontinuation, and the occurrence of elevations >3 times the upper limit of normal (ULN) in alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST), or elevations in bilirubin of >1.5 times the ULN. TEAEs of special interest were defined by pre-specified preferred terms for anemia, hepatic disorders, hypotension, edema, and fluid retention.

Exploratory pharmacokinetic (PK) endpoints for macitentan and its metabolite ACT-132577 were assessed. For the phase I and Ib studies, trough plasma concentrations were measured for all doses of macitentan on Days 1, 8, 14, on the last day of TMZ treatment at Months 2 and 6, and at progression or EOT. Samples for determining TMZ and monomethyl-triazeno-imidazole-carboxamide (MTIC) concentrations were collected 1 hour after TMZ administration on Day 14, on the last treatment day in Month 2 and Month 6, and at progression or EOT. Additionally, in the phase Ib study, a 24-hour PK profile for macitentan and ACT-132577 was obtained from patients receiving 150 mg macitentan on Day 14. For the ancillary study, trough plasma concentrations of macitentan and its metabolite (ACT-132577) were measured for all doses of macitentan on Day 1, Day 8, Day of surgery, on the last day of TMZ treatment at Months 2, 6, and at progression or EOT.

Exploratory efficacy endpoints were PFS, OS, time to progression (TTP), and objective response rate (ORR). Time to progression was defined as the time from treatment initiation until documentation of disease progression by contrast-enhanced magnetic resonance imaging, or until neurological deterioration. Tumor response was assessed by the MacDonald criteria,[18] using contrast-enhanced brain magnetic resonance imaging (MRI) and clinical assessment. Response criteria were defined as complete response, partial response, stable disease, or progression (Supplementary methods).

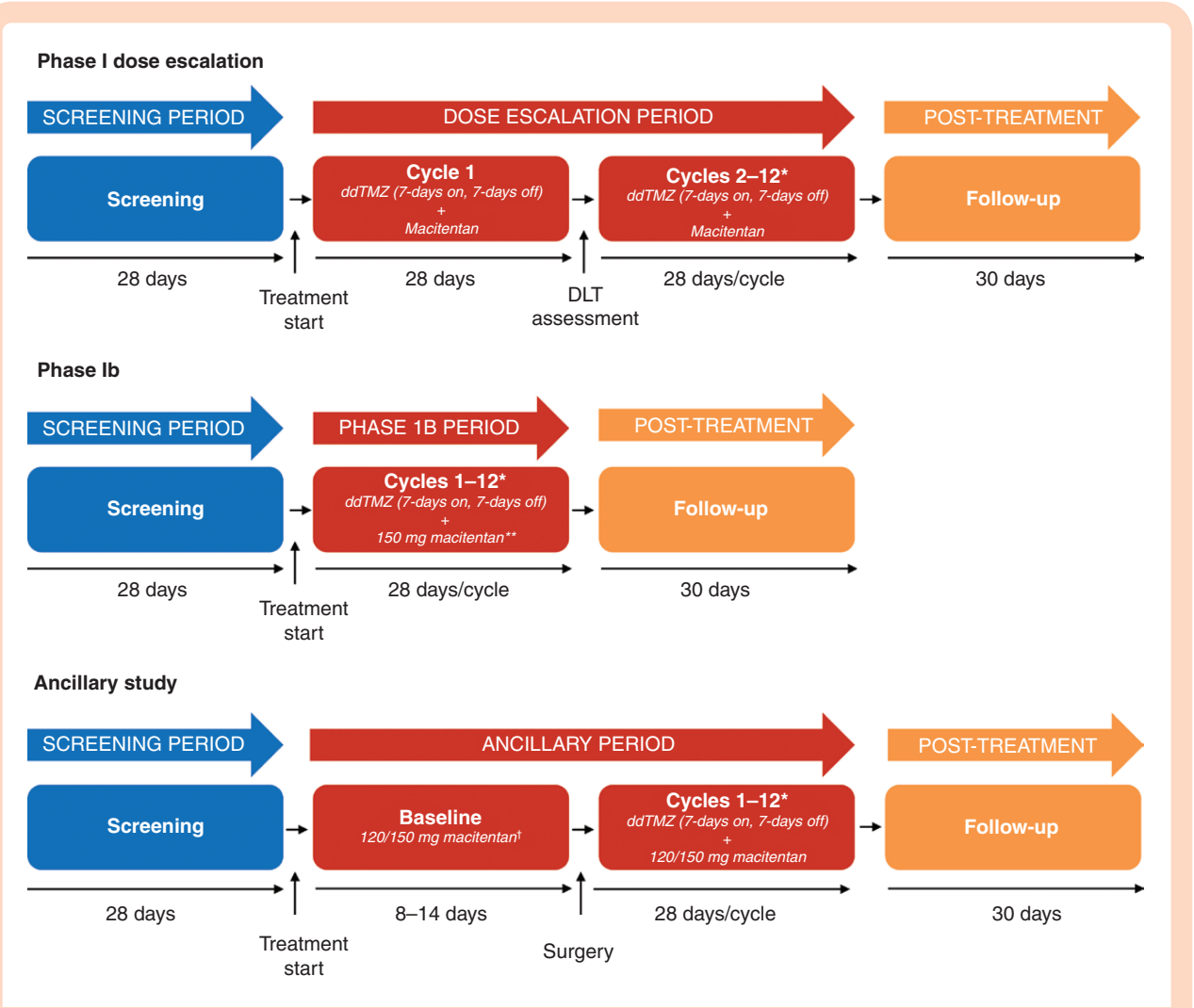

Figure 1. Study design. The phase I dose escalation period followed a conventional 3+3 dose escalation design (Supplementary methods). A 30-day follow-up period following discontinuation of the study treatment was included for assessment of adverse events/serious adverse events, vital signs, physical examination and concomitant medications. Dose adjustments of TMZ were permitted if required. *End of treatment occurred if study treatment was discontinued due to disease progression, death or for another reason. **Assessment of an additional dose was planned but not completed due to early termination. †Macitentan was stopped 1-day prior to surgery and restarted 3–5 weeks post-surgery. DLT: Dose-limiting toxicity.

Biomarker Immunofluorescent Staining

Tumor biopsies were embedded in standard embedding medium for frozen tissue specimens to ensure optimum cutting temperature (OCT) and stored at –80˚C. Tissue sections of 4 µm were prepared using a cryostat (Leica CM3050S), mounted on positively charged slides, air-dried for 5 minutes, and stored at –80˚C.

Immunofluorescent staining was performed on sections from primary and recurrent tumor samples. Briefly, sections were incubated with primary antibodies against ET-A (Santa Cruz), ET-B (Santa Cruz), phospho mitogen associated protein kinase (pMAPK) (Cell Signaling Technology) or phospho RAC-alpha serine/threonine-protein kinase (pAKT) (Cell Signaling Technology) overnight at 4˚C, washed 3 times in phosphate-buffered saline (PBS) and then incubated with their requisite fluorescently-labeled secondary antibodies for 1–2 hours at room temperature. Subsequently, slides were rinsed before counterstaining with the nucleic acid marker Hoechst 33342 (H3570, Invitrogen). Sections were mounted using a glycerol/PBS solution containing 0.1 M propyl gallate to minimize photobleaching.

For double staining of ET-A or ET-B receptors with phospho-serine (pSER); following staining for ET-A or ET-B receptors, as described above, sections were washed and incubated with blocking solution for 30 minutes at room temperature, prior to the addition of the primary antibody against pSER (BD Biosciences) overnight at 4˚C. Secondary antibody incubation, nucleic acid counterstain, and slide mounting were performed as previously described.

Staining for each biomarker was performed on slides prepared in duplicate. If results from these two slides did not match, additional replicates were performed on tissue

sections derived from a separate region of the tumor tissue, until matching results were obtained.

Images were captured using an Olympus BX-51 fluorescence microscope at 400X optical magnification. Images were processed using proprietary Olympus software. Reference clinical specimens from GBM control tissue, untreated with macitentan, were provided by Dr. Ken Aldape and used to evaluate the signal intensity of biopsy sections obtained in this study. Signal intensity was qualitatively evaluated by two suitably qualified independent persons.

Statistical Methods

Data analysis was performed using the all-treated set unless otherwise specified. The all-treated set included enrolled patients who received any study therapy (macitentan or ddTMZ). The efficacy analysis set included all patients who received macitentan or ddTMZ, excluding patients in the ancillary study who did not receive any study treatment post-surgery. The PK analysis set included all patients for whom at least one PK blood sample was taken. The PD analysis set included all patients from whom at least one ET-1 blood sample was taken.

All analyses were descriptive, and patients were summarized by macitentan dose level. PFS, OS, and TTP were summarized using the Kaplan-Meier method and reported with 95% confidence intervals (CIs). ORR is reported with 95% exact CIs calculated by the Clopper Pearson method.

Results

Baseline Patient Data

Between January 2012 and November 2015, 47 patients were screened and a total of 38 patients were enrolled across the three parts of the study (Figure 2) and treated with macitentan doses from 30 mg to 300 mg (one patient received 300 mg). The all-treated set and PK analysis set included 38 patients and the efficacy analysis set included 36 patients (two patients were excluded as they did not receive any study treatment after surgery in the ancillary

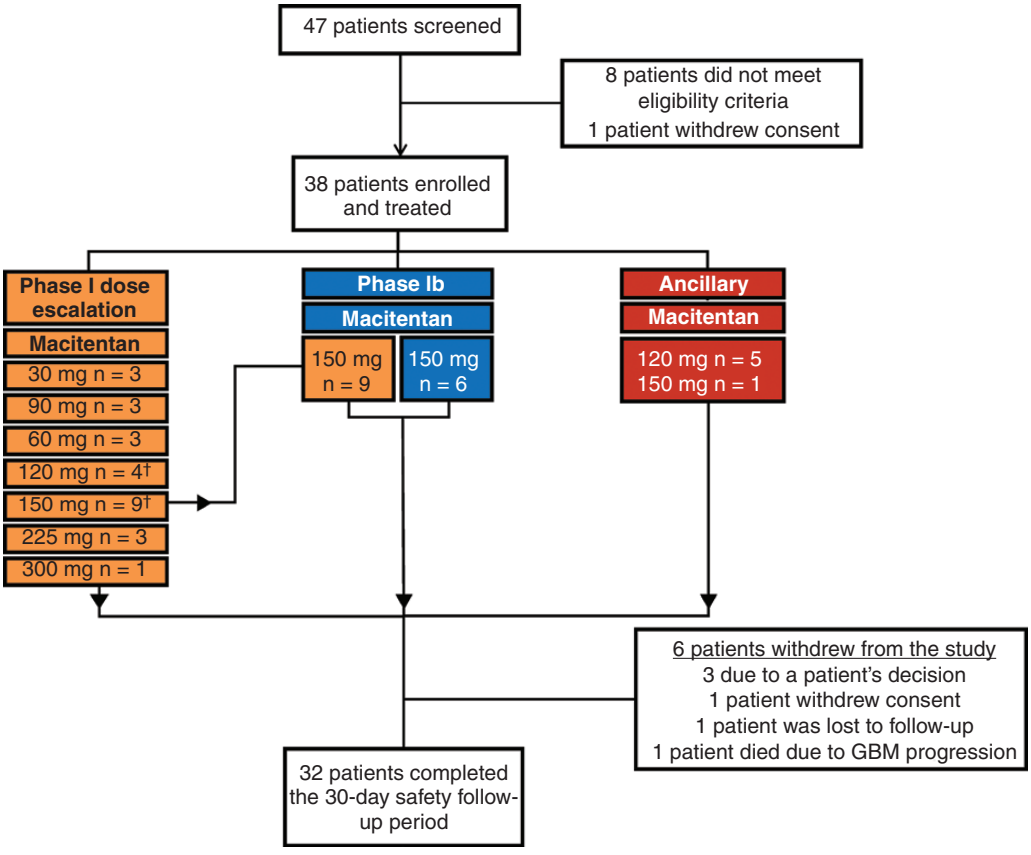

Figure 2. Patient disposition. The phase Ib study included 9 patients receiving 150 mg macitentan who were in the phase I dose escalation study and 6 additional patients that were not included in the phase I dose escalation study. Thirty-six patients discontinued study treatment before completing 12 cycles of treatment; 89.5% of early discontinuations were due to progressive disease. †1 patient in the 120 mg group and 3 patients in the 150 mg group were replaced due to incorrect treatment (Supplementary methods). Patients were contacted every 3 months until the end of the study to assess vital status.

study). Baseline patient demographics and characteristics by dose are described in Table 1.

The study was terminated early due to a sponsor decision, as described in the discussion. At the point of termination, the macitentan dose had been escalated to 300 mg.

Safety

Patients received macitentan for a median (range) duration of 61 (8–462) days; five (13.2%) patients received macitentan for more than six months and two (5.3%) patients for more than 12 months. Half of the patients received at least two cycles of ddTMZ in combination with macitentan and the median (range) duration of exposure to ddTMZ was 28 (7–171) days.

No DLTs were observed with macitentan doses up to 300 mg and an MTD was not determined. All patients had at least one TEAE, with lymphocyte count decrease (44.7%) most frequently reported (Table 2). No grade V TEAEs were reported. TEAEs of decreased lymphocyte count were the only reported Grade IV AEs (5 patients, 13.2%). TEAEs related to macitentan were reported for 16 (42.1%) patients and to ddTMZ treatment for 26 (68.4%) patients. A total of 17 (44.7%) patients had at least one SAE, none of which were related to macitentan. There were 12 patients (31.6%) who experienced a TEAE leading to discontinuation of study treatment (macitentan or both macitentan and ddTMZ). One patient died due to GBM progression deemed unrelated to macitentan use.

Eight (21.1%) patients had at least one TEAE of special interest; these were in the categories of edema and fluid retention (six patients; all unrelated to macitentan use) or hypotension (three patients; related to macitentan use for two patients). No AEs of treatment-emergent orthostatic hypotension (defined as ≥20 mmHg decrease in orthostatic systolic blood pressure or ≥10 mmHg decrease in orthostatic diastolic blood pressure) occurring from first dose up to 30 days after last dose were reported. No clinically meaningful changes in systolic or diastolic blood pressure were noted during the study. No TEAEs of special interest denoting anemia or hepatic disorders were reported. Sporadic non-dose-related elevations in ALT and/or AST of >1 to ≤3 × ULN, or elevations in bilirubin of >1 to ≤1.5 × ULN, were observed; however, these did not lead to discontinuation of macitentan.

Pharmacokinetics

Mean trough concentrations indicated that steady-state conditions for macitentan and ACT-132577 were reached by Week 1, with a dose-dependent increase in mean trough concentrations of both macitentan (from 338 ng/mL at 30 mg to 1960 ng/mL at 225 mg) and ACT-132577 (from 2633 ng/mL at 30 mg to 13610 ng/mL at 225 mg). Macitentan plasma concentrations did not reach a plateau over the dose range tested. Steady-state PK parameters following administration of macitentan 150 mg on Day 14 to six patients enrolled in the phase Ib study are summarized in Table 3, and 24-hour PK profiles are presented in Figure 3. There was no evidence for any mutual PK interaction between macitentan and TMZ (Supplementary Figure 2).

Exploratory Efficacy Analyses

Across all dose groups, the Kaplan-Meier median PFS and TTP estimate was 2.0 months (95% CI 1.6, 3.1); Kaplan-Meier estimate for percentage of patients alive and progression free at 6 months and 12 months was 16.7% (95% CI 6.8, 30.4) and 8.3% (95% CI 2.1, 20.1), respectively. Kaplan-Meier median overall survival in the 150 mg group was 9.3 months (95% CI 7.2, 10.5) and in all dose groups was 9.4 months (95% CI 8.5, 13.4). The best ORR (complete response plus partial response) was 13.9% (5 patients: 95% CI 4.7, 29.5) at both 6 and 12 months. Example MRI images from one of these patients with a partial response after 4 cycles of ddTMZ and 150 mg macitentan are shown in Supplementary Figure 3.

Ancillary Biomarker Analysis

Enrollment in the ancillary study was terminated at 6 patients due to lack of pre-treatment tumor samples and the substantial biomarker heterogeneity observed between patients and within tumors.

When clinical reference samples from untreated (control) patients were stained for ET-A/pSER, ET-B/pSER, pMAPK, and pAKT, a high degree of heterogeneity was observed between sections from the same patient, and between patients (Supplementary Figure 4). As such, it was not possible to establish a reference level of expression to use as a comparator for the samples collected from macitentan treated patients in the ancillary study.

Of the six patients enrolled in the ancillary study, data are presented for two patients treated with macitentan (120 or 150 mg), in which primary tumor tissue was available for comparison (Supplementary Figure 5). Data are not presented for the other four patients enrolled (who received macitentan 120): recurrent brain tumor tissue could not be obtained from two patients due to tissue necrosis, one patient died prior to surgery, and one patient did not have primary tissue available for comparison.

For two patients, presented in Supplementary Figure 5 biomarker expression in the primary, untreated tumor was compared with that in the post-macitentan treatment tumor sample obtained upon GBM recurrence. Post-treatment, immunofluorescent signals for ET-A/pSER, ET-B/pSER, MAPK, and AKT were low in comparison to primary tumor samples.

Discussion

The results of this phase I study show that macitentan was generally safe and well-tolerated in patients with GBM concomitantly receiving TMZ, at levels substantially higher than the 10 mg daily dose approved for PAH.[11]

Although all patients experienced at least one AE, the majority were associated with GBM and its complications, or otherwise the known side effects of TMZ. The AEs related to macitentan were consistent with its established safety profile.[11] Macitentan is a vasodilatory agent and AEs of special interest denoting hypotension were observed, however;

Table 1. Baseline Demographics and Disease Characteristics of Recurrent GBM Patient Population by Macitentan Dose

	Macitentan Dose							
	30 mg (*N* = 3)	60 mg (*N* = 3)	90 mg (*N* = 3)	120 mg (*N* = 9)*	150 mg (*N* = 16)*	225 mg (*N* = 3)	300 mg (*N* = 1)	Total (*N* = 38)
Age, years	54.0 (54–58)	42.0 (28–58)	61.0 (47–62)	52.0 (49–67)	54.5 (42–67)	52.0 (34–70)	65	54.0 (28–70)
Age distribution, *n* (%)								
18–64 years	3 (100)	3 (100)	3 (100)	8 (89)	12 (75)	2 (67)	0	31 (82)
65–84 years	0	0	0	1 (11)	4 (25)	1 (33)	1 (100)	7 (18)
Male sex, *n* (%)	2 (67)	3 (100)	0	5 (56)	12 (75)	2 (67)	1 (100)	25 (66)
Race, *n* (%)								
Caucasian/White	3 (100)	3 (100)	3 (100)	8 (89)	16 (100)	3 (100)	1 (100)	37 (97)
Hispanic	0	0	0	1 (11)	0	0	0	1 (3)
Time from histological diagnosis, weeks	63.4 (43–69)	45.6 (37–132)	62.1 (37–68)	38.7 (23–125)	45.9 (27–160)	61.0 (30–256)	27.3	44.7 (23–256)
Time from diagnosis of recurrence, weeks	3.3 (2–5)	1.1 (1–6)	1.9 (1–4)	2.0 (1–4)	2.9 (1–10)	5.1 (2–7)	12.4	2.5 (1–13)
Time after radiotherapy, weeks	51.0 (31-59)	31.0 (27-122)	51.0 (26-60)	27.0 (13-111)	36.0 (13-150)	54.0 (19-248)	21	32.5 (13–248)
Number of cycles of adjuvantTMZ treatment completed	8.0 (6–10)	6.0 (5–12)	11.0 (4–14)	3.0 (2–12)	5.5 (0–12)	10.0 (2–13)	2	6.0 (0–14)
MGMT status, *n* (%)[#]								
Methylated	1 (33)	1 (33)	0	2 (22)	5 (31)	1 (33)	0	10 (26)
Unmethylated	2 (67)	2 (67)	2 (67)	5 (56)	11 (69)	2 (67)	0	24 (63)

Values shown are median (range) unless otherwise stated.
*Includes five patients on macitentan 120 mg and one patient on 150 mg from the ancillary study.
[#]MGMT status could not be determined in four patient samples.
GBM: glioblastoma; MGMT: O6-methylguanine-DNA-methyltransferase; TMZ: temozolomide.

Table 2. Treatment Emergent Adverse Events in Recurrent GBM Patients

	Macitentan Dose							
	30 mg (*N* = 3)	60 mg (*N* = 3)	90 mg (*N* = 3)	120 mg (*N* = 9)*	150 mg (*N* = 16)*	225 mg (*N* = 3)	300 mg (*N* = 1)	Total (*N* = 38)
Patients with ≥1 treatment emergent adverse event, *n* (%)	3 (100.0)	3 (100.0)	3 (100.0)	9 (100.0)	16 (100.0)	3 (100.0)	1 (100)	38 (100.0)
Treatment emergent adverse events by CTCAE grade, *n* (%)	Grade 3, 3 (100)	Grade 3, 2 (67) Grade 2, 1 (33)	Grade 3, 3 (100)	Grade 4, 1 (11) Grade 3, 5 (56) Grade 2, 2 (22) Grade 1, 1 (11)	Grade 4, 3 (19) Grade 3, 10 (63) Grade 2, 2 (13) Grade 1, 1 (6)	Grade 4, 1 (33) Grade 2, 1 (33) Grade 1, 1 (33)	Grade 4, 1 (100)	Grade 4, 5 (13) Grade 3, 24 (63) Grade 2, 6 (16) Grade 1, 3 (8)
Treatment emergent adverse event, *n* (%)†								
Lymphocyte count decreased	2 (67)	1 (33)	0	3 (33)	9 (56)	2 (67)	0	17 (45)
Glioblastoma	0	2 (67)	1 (33)	3 (33)	7 (44)	0	0	13 (34)
Headache	2 (67)	2 (67)	1 (33)	1 (11)	5 (31)	2 (67)	0	13 (34)
Seizure	1 (33)	2 (67)	1 (33)	2 (22)	5 (31)	0	1 (100)	12 (32)
Fatigue	3 (100)	1 (33)	1 (33)	2 (22)	1 (6)	2 (67)	0	10 (26)
Constipation	0	0	1 (33)	4 (44)	2 (13)	2 (67)	0	9 (24)
Nausea	1 (33)	1 (33)	1 (33)	3 (33)	2 (13)	1 (33)	0	9 (24)
Platelet count decreased	1 (33)	1 (33)	0	1 (11)	4 (25)	0	0	7 (18)
Vomiting	0	1 (33)	1 (33)	1 (11)	3 (19)	1 (33)	0	7 (18)
Dizziness	2 (67)	1 (33)	1 (33)	0	1 (6)	0	0	5 (13)
Neutrophil count decreased	0	0	0	1 (11)	3 (19)	1 (33)	0	5 (13)
White blood cell count decreased	0	0	0	1 (11)	3 (19)	1 (33)	0	5 (13)
Diarrhea	1 (33)	0	2 (67)	0	1 (6)	0	0	4 (11)
Insomnia	0	0	1 (33)	1 (11)	1 (6)	1 (33)	0	4 (11)
Lymphopenia	0	1 (33)	3 (100)	0	0	0	0	4 (11)
Nasal congestion	0	0	2 (67)	1 (11)	1 (6)	0	0	4 (11)
Edema peripheral	0	0	0	1 (11)	3 (19)	0	0	4 (11)
Rash maculo-papular	0	0	1 (33)	0	2 (13)	1 (33)	0	4 (11)
Aphasia	1 (33)	1 (33)	0	1 (11)	0	0	0	3 (8)
Confusional state	0	0	0	2 (22)	1 (6)	0	0	3 (8)
Hemiparesis	1 (33)	0	0	0	1 (6)	0	0	3 (8)
Hypotension	0	1 (33)	0	1 (11)	1 (6)	0	0	3 (8)
Muscular weakness	1 (33)	0	0	1 (11)	1 (6)	0	0	3 (8)
Sinusitis	1 (33)	0	0	1 (11)	1 (6)	0	0	3 (8)

Table 2. Continued

	Macitentan Dose							
	30 mg (*N* = 3)	60 mg (*N* = 3)	90 mg (*N* = 3)	120 mg (*N* = 9)*	150 mg (*N* = 16)*	225 mg (*N* = 3)	300 mg (*N* = 1)	Total (*N* = 38)
Brain edema	0	0	0	1 (11)	1 (6)	0	0	2 (5)
Gastroesophageal reflux disease	1 (33)	0	0	0	1 (6)	0	0	2 (5)
Oropharyngeal pain	0	0	0	1 (11)	1 (6)	0	0	2 (5)
Pyrexia	0	0	0	1 (11)	1 (6)	0	0	2 (5)
Urinary tract infection	0	0	0	0	2 (13)	0	0	2 (5)
VIIth nerve paralysis	0	0	0	0	2 (13)	0	0	2 (5)
Weight decreased	0	0	0	1 (11)	1 (6)	0	0	2 (5)

*Includes 5 patients on macitentan 120 mg and 1 patient on 150 mg from the ancillary study.
†Adverse events occurring in ≥5% of the total patient population. Adverse events were coded in accordance with the Medical Dictionary for Regulatory Activities, version 18.0.
CTCAE: Common Terminology Criteria for Adverse Events.

none of these met the criteria for orthostatic hypotension. No clinically relevant shifts in liver function tests were observed at any dose level despite the hepatotoxic nature of TMZ. No transient ALT elevations exceeded >3 × ULN and no bilirubin elevations exceeded >1.5 x ULN; all enzyme levels returned to normal or pre-treatment levels while patients remained on macitentan. No novel safety signals related to fluid retention or anemia were observed at doses higher than 10 mg of macitentan. Despite concurrent treatment with TMZ and macitentan, both of which can increase the risk of hepatic and anemia-related adverse events,[11,19] the rates of events in this study were consistent with those seen in patients receiving either agent individually, suggesting no additive or synergistic effect on these safety outcomes.

PK analysis indicated that macitentan reached steady-state within 1 week, which is consistent with data generated in healthy volunteers.[14] The median t_{max} of macitentan 150 mg and ACT-132577 (measured on Day 14) was 5.5 hours post-dosing, earlier than the 10-hour (at Day 1) and 8-hour (Day 10) median t_{max} observed in healthy individuals receiving doses of macitentan up to 30 mg.[14] No plateau in macitentan exposure was observed over the dose range tested in this study. A previous single-ascending dose study in healthy volunteers[15] reported a non-proportional increase in macitentan C_{max} over a dose range of 0.2–600 mg. Consistent with the results reported here, no plateau in macitentan plasma concentration was reported.[15] This is also consistent with observations from another multiple ascending dose study in healthy individuals with up to 30 mg macitentan.[14]

In the current study, treatment with macitentan and ddTMZ led to an ORR of 13.9% and median OS of 9.4 months; in pooled analyses of multiple clinical trials for recurrent GBM, ORRs ranged from 4–7% and median OS was 5–7 months.[20] Although a subsequent clinical trial reported an ORR of 41.5% and median OS of 9.1 months for patients treated with lomustine plus bevacizumab,[21] the discrepancy in progression reported by central review and local assessment may make these values less reliable; this was not discussed by the authors. Furthermore, the ORR for the lomustine alone arm in this trial was 13.9%,[21] which is considerably higher than the ORR reported in other trials.[6,22] It should be noted that the evaluation of efficacy presented here was exploratory and there were too few patients for any clinically meaningful conclusions to be drawn. Although the study was not powered to establish efficacy, the publication of supportive preclinical data led to the anticipation that a stronger efficacy signal would be observed. As such, the sponsor made the decision to prematurely terminate the study.

The findings in this trial could be impacted by several elements. It is not known whether macitentan crosses the blood-brain barrier (BBB); macitentan has greater lipophilicity than other ERAs, which may theoretically aid crossing of the BBB,[23] and the efficacy of macitentan in preclinical GBM models[12] suggests that, in mice, macitentan may cross the BBB. However, species differences may reduce macitentan BBB penetration in humans, alter macitentan pharmacokinetics, or account for differences in ET-A/ET-B receptor expression within

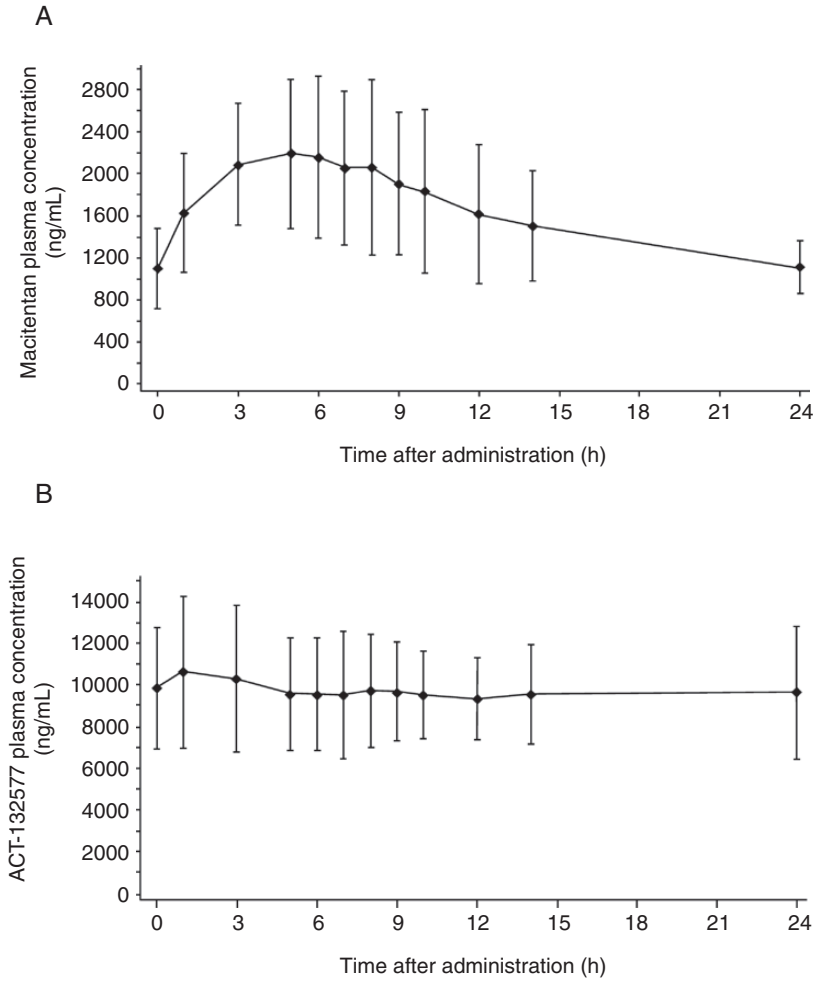

Figure 3. Macitentan and ACT-132577 plasma concentrations over 24 hours at steady state in the recurrent GBM patient population. (a) 24-hour PK profile for plasma macitentan concentrations after 150 mg macitentan at Day 14. *N* = 6 at each timepoint except *N* = 5 at 24 h. (b) 24-hour PK profile for plasma ACT-132577 concentrations after 150 mg macitentan at Day 14. *N* = 6 at each timepoint, except *N* = 5 at 24h. Data at each timepoint are mean ± SD.

Table 3. Summary of Plasma Macitentan and ACT-132577 PK Parameters Following Administration of 150 mg Macitentan

	C_{max} (ng/mL) *N* = 6	t_{max} (h) *N* = 6	*AUC*τ (h × ng/mL) *N* = 5
Macitentan	2334.2 (1788.3, 3046.7)	5.5 (3.0–10.0)	38862.5 (26926.5, 56089.6)
ACT-132577	10939.7 (7839.4, 15266.2)	5.5 (1.0–10.0)	222102.2 (149755.9, 329398.5)

Geometric means (95% confidence intervals) are given for all PK parameters except t_{max}, where median (range) are given.
*AUC*τ: Area under the plasma concentration-time curve over one dosing interval; GBM: glioblastoma; C_{max}: maximum plasma concentration; t_{max}: time to reach the maximum plasma concentration.

tumors, leading to a variable impact from endothelin receptor antagonism. Images from the ancillary study indicate lower ET-A and ET-B receptor phosphorylation (activation) in the macitentan-treated tumor samples in comparison to the untreated primary tumor; however, given the substantial variability in the staining for these markers in the control tissue, even within tumors from the same patient, this result may be independent of macitentan treatment, and no meaningful interpretation of this finding is possible.

The small number of patients enrolled in this study represents a key limitation. As recruitment may have been affected by increased interest in the development of new experimental immunotherapies for GBM, the chances of recruiting sufficient patients were believed to have been diminished. Lack of support for an efficacy signal from the ancillary biomarker study further contributed to the decision to terminate the study early.

The novelty of the current study lies in the investigation of a dual ERA alongside the cytotoxic agent TMZ in patients with recurrent GBM. Macitentan was determined to be generally safe and well tolerated in GBM patients at doses up to 30 times greater than the 10 mg once daily approved for PAH treatment. Combining macitentan with ddTMZ did not produce any new safety signals for either drug in GBM patients. Further studies to explore the potential biological mechanisms of macitentan are needed to understand the therapeutic potential of this agent against gliomas.

Supplementary Material

Supplementary data are available at *Neuro-Oncology Advances* online.

Keywords

glioblastoma | macitentan | phase I | recurrent | temozolomide

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References

1. Polivka J, Holubec L, Kubikova T, et al. Advances in experimental targeted therapy and immunotherapy for patients with glioblastoma multiforme. *Anticancer Res.* 2017;37(1):21–33.
2. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma–are we there yet? *Neuro Oncol.* 2013;15(1):4–27.
3. Alifieris C, Trafalis DT. Glioblastoma multiforme: pathogenesis and treatment. *Pharmacol Ther.* 2015;152:63–82.
4. Weller M, van den Bent M, Tonn JC, et al. European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. *Lancet Oncol.* 2017;18(6):e315–e329.

5. Cohen MH, Shen YL, Keegan P, Pazdur R. FDA drug approval summary: bevacizumab (avastin) as treatment of recurrent glioblastoma multiforme. *Oncologist.* 2009;14(11):1131–1138.
6. Weller M, Le Rhun E. How did lomustine become standard of care in recurrent glioblastoma? *Cancer Treat Rev.* 2020;87:102029.
7. Nagane M. Dose-dense temozolomide: is it still promising? *Neurol Med Chir (Tokyo).* 2015;55 Suppl 1:38–49.
8. Vasaikar S, Tsipras G, Landázuri N, et al. Overexpression of endothelin B receptor in glioblastoma: a prognostic marker and therapeutic target? *BMC Cancer.* 2018;18(1):154.
9. Egidy G, Eberl LP, Valdenaire O, et al. The endothelin system in human glioblastoma. *Lab Invest.* 2000;80(11):1681–1689.
10. Bagnato A, Rosanò L. The endothelin axis in cancer. *Int J Biochem Cell Biol.* 2008;40(8):1443–1451.
11. Opsumit® (macitentan). *Full Prescribing Information.* Actelion Pharmaceuticals US, Inc. March. Updated 2019. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/204410s017lbl.pdf
12. Kim SJ, Lee HJ, Kim MS, et al. Macitentan, a dual endothelin receptor antagonist, in combination with Temozolomide Leads to Glioblastoma regression and long-term survival in mice. *Clin Cancer Res.* 2015;21(20):4630–4641.
13. Kim SJ, Kim JS, Kim SW, et al. Macitentan (ACT-064992), a tissue-targeting endothelin receptor antagonist, enhances therapeutic efficacy of paclitaxel by modulating survival pathways in orthotopic models of metastatic human ovarian cancer. *Neoplasia.* 2011;13(2):167–179.
14. Sidharta PN, van Giersbergen PL, Dingemanse J. Safety, tolerability, pharmacokinetics, and pharmacodynamics of macitentan, an endothelin receptor antagonist, in an ascending multiple-dose study in healthy subjects. *J Clin Pharmacol.* 2013;53(11):1131–1138.
15. Sidharta PN, van Giersbergen PL, Halabi A, Dingemanse J. Macitentan: entry-into-humans study with a new endothelin receptor antagonist. *Eur J Clin Pharmacol.* 2011;67(10):977–984.
16. Tolcher AW, Gerson SL, Denis L, et al. Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br J Cancer.* 2003;88(7):1004–1011.
17. Le Tourneau C, Lee JJ, Siu LL. Dose escalation methods in phase I cancer clinical trials. *J Natl Cancer Inst.* 2009;101(10):708–720.
18. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol.* 1990;8(7):1277–1280.
19. Merck & Co. Temozolomide product information. https://www.merck.com/product/usa/pi_circulars/t/temodar_capsules/temodar_pi.pdf. Updated October 2017.
20. Colman H. Future directions in glioblastoma therapy. *Am Soc Clin Oncol Educ Book.* 2012:108–111. doi:10.14694/EdBook_AM.2012.32.108
21. Wick W, Gorlia T, Bendszus M, et al. Lomustine and bevacizumab in progressive glioblastoma. *N Engl J Med.* 2017;377(20):1954–1963.
22. Batchelor TT, Mulholland P, Neyns B, et al. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J Clin Oncol.* 2013;31(26):3212–3218.
23. Pollock DM, Boesen EI, Black SM. Does targeting the lipophilic milieu provide advantages for an endothelin antagonist? *Mol Interv.* 2009;9(2):75–78.