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Phase 1 dose-escalation study of the antiplacental growth factor monoclonal antibody RO5323441 combined with bevacizumab in patients with recurrent glioblastoma

Ulrik Lassen, Olivier L. Chinot, Catherine McBain, Morten Mau-Sørensen, Vibeke Andrée Larsen, Maryline Barrie, Patrick Roth, Oliver Krieter, Ka Wang, Kai Habben, Jean Tessier, Angelika Lahr, and Michael Weller

Department of Oncology, Rigshospitalet, Copenhagen, Denmark (U.L., M.M.-S.); Department of Radiology, Rigshospitalet, Copenhagen, Denmark (V.A.L.); Aix-Marseille University A.P.-H.M., Department of Neuro-Oncology, University Hospital Timone, Marseille, France (O.L.C., M.B.); Department of Clinical Oncology, The Christie Hospital N.H.S Foundation Trust, Manchester, England (C.M.); Department of Neurology, University Hospital Zurich, Zurich, Switzerland (P.R., M.W.); Roche Diagnostics GmbH, Penzberg, Germany (O.K., K.H., A.L.); Hoffmann La Roche Pharmaceuticals, Nutley, NewJersey (K.W.); F. Hoffmann-La Roche Ltd, Basel, Switzerland (J.T.)

Corresponding Author: Ulrik Lassen, MD, PhD, Department of Oncology 5072, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark (ulrik.lassen@rh.regionh.dk).

Background. We conducted a phase 1 dose-escalation study of RO5323441, a novel antiplacental growth factor (PlGF) monoclonal antibody, to establish the recommended dose for use with bevacizumab and to investigate the pharmacokinetics, pharmacodynamics, safety/tolerability, and preliminary clinical efficacy of the combination.

Methods. Twenty-two participants with histologically confirmed glioblastoma in first relapse were treated every 2 weeks with RO5323441 (625 mg, 1250 mg, or 2500 mg) plus bevacizumab (10 mg/kg). A standard 3 + 3 dose-escalation trial design was used.

Results. RO5323441 combined with bevacizumab was generally well tolerated, and the maximum tolerated dose was not reached. Two participants experienced dose-limiting toxicities (grade 3 meningitis associated with spinal fluid leak [1250 mg] and grade 3 cerebral infarction [2500 mg]). Common adverse events included hypertension (14 participants, 64%), headache (12 participants, 55%), dysphonia (11 participants, 50%) and fatigue (6 participants, 27%).The pharmacokinetics of RO5323441 were linear, over-the-dose range, and bevacizumab exposure was unaffected by RO5323441 coadministration. Modulation of plasmatic angiogenic proteins, with increases in VEGFA and decreases in FLT4, was observed. Dynamic contrast-enhanced/diffusion-weighted MRI revealed large decreases in vascular parameters that were maintained through the dosing period. Combination therapy achieved an overall response rate of 22.7%, including one complete response, and median progression-free and overall survival of 3.5 and 8.5 months, respectively.

Conclusion. The toxicity profile of RO5323441 plus bevacizumab was acceptable and manageable. The observed clinical activity of the combination does not appear to improve on that obtained with single-agent bevacizumab in patients with recurrent glioblastoma.

Keywords: bevacizumab, dose-escalation study, glioblastoma, placental growth factor, RO5323441.

Tumor angiogenesis is a fundamental step in carcinogenesis,[1] and the vascular endothelial growth factor (VEGF) family of glycoproteins (VEGFA–D [P1GF] and placental growth factor [PGF/PlGF]) are central to this process.[2,3] PlGF is a pleiotropic, proangiogenic growth factor that can stimulate tumor angiogenesis directly by affecting endothelial cells, pericytes, and smooth muscle cells as well as indirectly by enhancing VEGF-driven angiogenesis and by attracting proangiogenic myeloid cells.[4] PlGF expression is increased in some tumor types and can correlate with tumor stage and poor outcomes, including disease progression and reduced survival.[5–7]

Glioblastoma is the most aggressive malignant primary brain tumor in adults and is a primary target for antiangiogenic therapy due to the high degree of vascularization.[8,9] Several VEGF pathway-targeting drugs have emerged including cediranib, aflibercept, and the anti-VEGF monoclonal antibody

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bevacizumab, which is both active and well tolerated in patients with glioblastoma. Based on the durable objective response rates (ORRs) demonstrated in two phase 2 trials[10,11] in 2009, the US Food and Drug Administration granted accelerated approval of single-agent bevacizumab for the treatment of patients with progressive glioblastoma following prior therapy.[12] However, eventual disease progression is inevitable because of the emergence of resistance to VEGF and VEGR receptor (VEGFR/KDR)-directed therapy.[13]

Simultaneous blockade of multiple angiogenic pathways might improve efficacy and reduce therapeutic resistance. Evidence indicates that PlGF is upregulated in response to VEGF(R) inhibition, supporting the development of combined therapy that targets both VEGFA and PlGF.[14,15] RO5323441 (RG7334/TB-403) is a humanized recombinant immunoglobulin G_1 (IgG_1) anti-PlGF monoclonal antibody. Unlike VEGF, PlGF selectively binds VEGFR1 (FLT1) and its co-receptors neuropilin-1 (NRP1) and -2 (NRP2).[16] Preclinical studies have shown that RO5323441 inhibits the growth of VEGF blockade-resistant tumors without affecting healthy vessels.[16] RO5323441 is also capable of enhancing the efficacy of chemotherapy and VEGFR inhibitors in vivo and inhibiting angiogenesis and tumor cell motility.[16,17] Single-dose RO5323441 displayed a favorable safety profile and dose-linear pharmacokinetics (PK) in a first-in-human dose-escalation trial.[18] A subsequent phase 2 study of 23 patients with advanced solid tumors showed that RO5323441 was well tolerated up to a dose of 30 mg/kg every 3 weeks. No dose-limiting toxicities (DLTs) were reported, and hence the maximum tolerated dose (MTD) was not reached.[19] PK analysis confirmed the dose-proportional exposure of RO5323441 with a terminal half-life of 9–14 days.

Here we report the results of a phase 1 study that investigated RO5323441 combined with bevacizumab in patients with recurrent glioblastoma. The primary objective was to establish the recommended dose of RO5323441 for use with bevacizumab. Secondary objectives included evaluation of the PK, pharmacodynamics (PD), safety/tolerability, and preliminary efficacy of the combination treatment.

Methods

Study Design

Study NCT01308684 was a phase 1b, open-label, dose-escalation multicenter study of RO5323441 combined with bevacizumab in patients with recurrent glioblastoma. The study was conducted at 4 centers in Switzerland, France, Denmark, and the United Kingdom. Local ethics committee approval was obtained, and all participants were able to provide their own written informed consent. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

From day 1, 3 fixed doses of RO5323441 (625, 1250, and 2500 mg) were administered once every 2 weeks (q2w) with 10 mg/kg bevacizumab. A standard $3+3$ dose-escalation study design was used to determine the MTD based on the occurrence of DLTs. At the MTD or top dose level (set at 2500 mg), 6 additional participants were enrolled into an expansion cohort to further evaluate safety/tolerability, PK, and PD. This cohort received pretreatment with single-agent bevacizumab (10 mg/kg) on day −14 and day 1, single-agent RO5323441 (2500 mg) on day −2 with combination therapy given q2w from day 15 onwards. This dosing schedule was selected to allow us to first measure the PD effects of bevacizumab monotherapy, after which any additional PD effect due to combination dosing with RO5323441 could be determined. Participants continued treatment until disease progression, unacceptable toxicity, investigator decision, or patient refusal.

Patients

Eligible patients were ≥18 years of age with histologically confirmed glioblastoma in first relapse and radiographic evidence of disease progression. Patients had already received standard frontline radiotherapy and temozolomide (TMZ) and had a Karnofsky performance status ≥70%, adequate hematological (absolute neutrophil count ≥1 500/μL; platelets ≥100 000/μL), and hepatic and renal function. Following radio-chemotherapy, a minimum treatment interval of 12 weeks was required to reduce the likelihood of pseudoprogression. Participants receiving corticosteroids had to be on a stable or decreasing dose for ≥5 days before a baseline MRI scan was conducted.

Exclusion criteria included previous treatment with PlGF/VEGF(R) targeting agents, cilengitide, enzastaurin, or intracerebral agents; MRI evidence of recent brain hemorrhage, uncontrolled arterial hypertension or prior history of hypertensive crisis/encephalopathy; prior bleeding diathesis or coagulopathy; major surgery and hemoptysis within 1 month; or a history of significant cerebrovascular/cardiovascular disease, abdominal fistula, gastrointestinal perforation, or intracranial abscess within 6 months.

Study Drug Administration

RO5323441 and bevacizumab were administered as continuous intravenous (IV) infusions. RO5323441 was administered immediately prior to bevacizumab.

Safety Assessments

The participants were seen before each study drug administration and weekly during the first 4 weeks. Safety assessments included physical (performance status, vital signs) and laboratory examinations as well as twice-daily measurement of blood pressure for the first 4 weeks. Baseline and end-of-study assessments included electrocardiograms (ECGs) and lower extremity ultrasounds. Adverse events (AEs) were defined according to the NCI Common Terminology Criteria for AEs (CTCAE), version 4.0.[20]

Definition of Dose-limiting Toxicity and Maximum Tolerated Dose

A DLT was defined as a study drug-related CTCAE that occurred during the first 28 days of treatment with RO5323441 plus bevacizumab. These included: grade (G) 4 neutropenia or thrombocytopenia; G3 thrombocytopenia with bleeding; febrile neutropenia and/or documented infection requiring IV antibiotics; any nonhematological G4 event or G3 event that caused a dosing delay of >7 days (except for G3 nausea/vomiting, diarrhea, and skin AEs without adequate supportive care measures);

or any recurrence of non-DLT G3 nonhematological event. Additional protocol-specific DLTs were G4 fistula; ≥G3 cardiac disorder; hemorrhage or hypertension if uncontrolled with medication; ≥G2 thromboembolic event or pulmonary hemorrhage; or any grade intracranial hemorrhage, gastrointestinal perforation, tracheo-esophageal fistula, or reversible posterior leukoencephalopathy. The MTD was defined as the dose level below any dose level with more than one DLT.

Pharmacokinetic Assessments

For the dose-escalation part of the study, PK blood samples were collected before and after each infusion with additional samples obtained on days 8 and 22 (7 days after the first 2 infusions). For the expansion cohort, blood was collected before and after the infusions on days −14 and 1 (bevacizumab only), day −2 (RO5323441 only), and at all other dosing occasions (combination treatment). Additional samples were obtained on days 8 and 22. Except for day −14, preinfusion samples were taken <3 hours prior to the dynamic contrast-enhanced (DCE)-MRI scan. Serum-free bevacizumab concentrations were determined by ELISA using recombinant human VEGF for capture and goat antihuman IgG conjugated to horseradish peroxidase for detection, as described previously.[21] RO5323441 quantification was performed using a sandwich ELISA with immobilized biotinylated recombinant human PlGF as capture reagent and digoxigenylated (F(ab')2-specific) sheep polyclonal anti-RO5323441 antibody with horseradish peroxidase-conjugated antidigoxigenin Fab fragments as detection reagents. Only RO5323441 with free and active binding sites was detected with the analytical method.

Pharmacodynamic Assessments

For the expansion cohort only, the pharmacological effect of RO5323441 on vascular parameters was investigated by DCE-MRI and diffusion-weighted (DW)-MRI conducted twice at baseline and on days −2, 1, 15, and 53–55 (Fig. 1A). Image acquisition was performed before, during, and after the i.v. administration of a gadolinium contrast agent while participants were on a stable dose of steroids for at least 5 days. DCE-MRI was conducted before any study drug treatment scheduled for the same day. Image acquisition was performed locally, and analysis of the scans was conducted centrally.

Plasma PlGF, VEGFA, and fms-related tyrosine kinase-4 (FLT4) levels were quantified using immunological multiparametric chip technique (IMPACT) analysis as part of a Roche solid phase antibody sandwich ELISA. Blood was collected on days 1, 8, 15, 29, 53–55, 85, and every 3 months thereafter, with additional samples on days −14 and −2 in the expansion cohort only. For VEGFA, this assay detects both free VEGFA and part of the bound VEGFA.

Efficacy Assessments

Tumor response was evaluated every 8 weeks using Revised Assessment in Neuro-Oncology (RANO) criteria,[22] which combine radiological tumor assessment with neurological assessment while taking into account corticosteroid use. Disease imaging was performed locally, and scans were interpreted by a single radiologist at each site wherever possible. Best ORR, disease control rate (DCR), median progression-free survival (PFS), 6-month PFS rate (PFS-6), and median overall survival (OS) were calculated. DCR was defined as the rate of combined complete responses, partial responses, and stable disease as assessed by RANO criteria.

Statistical Considerations

All participants who received at least one dose of study medication were included in the safety and efficacy populations. DCE-/DW-MRI parameters were evaluated using a within-patient change from baseline. Median time-to-event for PFS and OS was analyzed using Kaplan-Meier estimates.

Results

Participant Characteristics and Treatment

Twenty-two participants were enrolled into 3 dose groups (Table 1). All participants had received previous TMZ treatment with radiotherapy after surgery. All participants received 10 mg/kg bevacizumab. Cumulative RO5323441 doses ranged from 2.5 g to 60 g. Participants received a median of 8 doses (range, 1–27 doses) of both RO5323441 and bevacizumab. Sixteen participants discontinued the study due to progressive disease, one withdrew consent, and 2 patients discontinued the study due to DLTs. Three other participants were withdrawn by the investigator after complete metabolic responses on ^{18F}-FDG PET scans after they had experienced objective anatomic responses (2 complete responses, one partial response) for up to 18 months while on study treatment. These participants were censored in PFS and OS analyses.

Safety

Two DLTs were reported in 2 participants. One participant experienced G3 meningitis associated with cerebrospinal fluid (CSF) leak 12 days after the second dose of RO5323441 (1250 mg) and bevacizumab. This participant had undergone surgery to resect the right uncus 1 month prior to first dose of study drug. The epidural/subgaleal CSF leakage observed at the time of meningitis was first noted 6 weeks previously, and it was believed that bevacizumab might have caused or compromised regression of preexisting CSF leakage that enhanced the risk of infection. The second DLT (G3 cerebral infarction) occurred 5 days after the first dose of combination therapy (2500 mg RO5323441). No MTD was reached, and the highest dose of RO5323441 tested was 2500 mg. Sixteen (73%) participants died due to disease progression following study discontinuation, whereas the remaining participants were alive.

One hundred forty-six AEs were reported in 21 participants (Table 2) including 31 G3/4 AEs (in 17 participants). Fifty-five study drug-related AEs occurred in 20 participants including hypertension (13 participants, 59%), dysphonia (11 participants, 50%), epistaxis (4 participants,18%) and fatigue (3 participants, 14%). The incidence and severity of AEs and drug-related AEs were similar across dosing groups. Of the 21serious AEs (SAEs) reported in 13 participants, only hypertension,

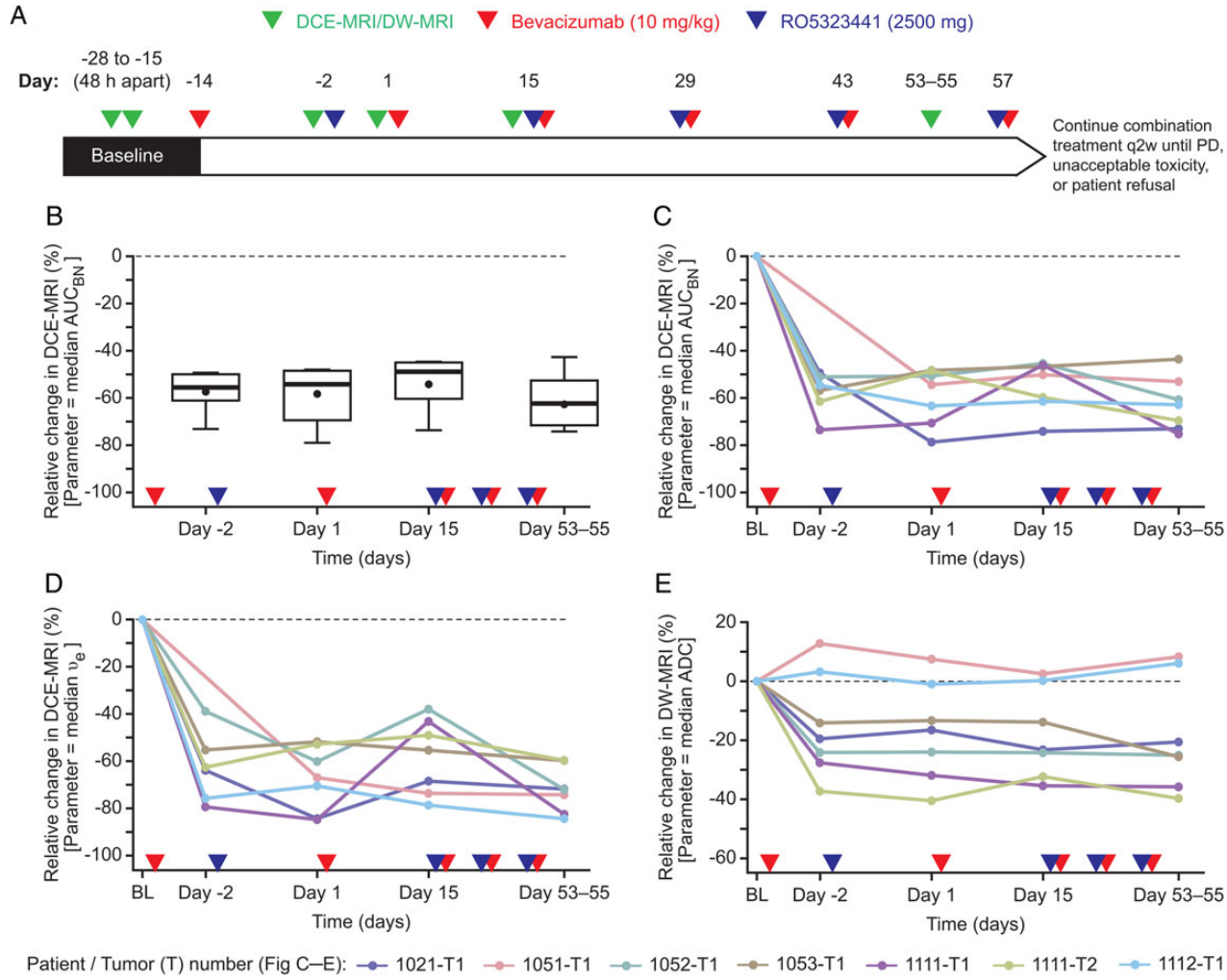

Fig. 1. Effect of antiangiogenic treatment on DCE-MRI and DW-MRI parameters for the 6 participants (7 tumors) in the expansion cohort. DCE-MRI/DW-MRI was performed twice at baseline and prior to any scheduled study drug on day −2 (2 weeks after the first dose of single-agent bevacizumab), day 1, day 15, and days 53–55 (A). Overall relative change from baseline in median AUC_{BN} (B) and individual profiles for the relative change from baseline in median AUC_{BN} (C), median v_e (D), and median ADC (E) are indicated. Abbreviations: ADC, water diffusion coefficient; AUC_{BN}, area under the gadolinium concentration curve normalized with plasma input function; BL, baseline; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; DW-MRI, diffusion-weighted magnetic resonance imaging; PD, progressive disease; q2w, once every 2 weeks; v_e, fractional extracellular extravascular volume.

Table 1. Patient characteristics ($N = 22$)

Parameter	RO5323441 Dose			
	625 mg ($N = 4$)	1250 mg ($N = 6$)	2500 mg ($N = 12$[a])	All ($N = 22$)
Sex, *n* (%)				
Male	2 (50)	5 (83)	9 (75)	16 (73)
Female	2 (50)	1 (17)	3 (25)	6 (27)
Age, median (range)	60.0 (50–66)	58.0 (41–69)	57.0 (37–72)	58.0 (37–72)
Weight in kg, median (range)	73.0 (48.6–92.8)	83.5 (69.4–95.0)	75.0 (62.0–98.0)	79.5 (48.6–98.0)
BMI in kg/m^2, median (range)	24.4 (18.8–26.3)	26.4 (22.7–31.3)	25.6 (20.0–31.4)	25.7 (18.8–31.4)
Corticosteroid use *n* (%)	2 (50)	3 (50)	11 (92)	16 (73)

Abbreviation: BMI, body mass index
[a]Includes patients from both the dose-escalation ($n = 6$) and expansion cohort parts ($n = 6$) of the study.

Table 2. Adverse events of any grade reported by >10% of the study population and all G 3/4 events

Event	Patients by Treatment Group and AE Grade (*n* [%])			
	625 mg ($N=4$)	1250 mg ($N=6$)	2500 mg ($N=12$)	All ($N=22$)
AE leading to discontinuation	–	1 (17)	1 (8)	2 (9)
SAE	1 (25)	5 (83)	7 (58)	13 (59)
Any grade AE in >10% patients				
Hypertension	3 (75)	3 (50)	8 (67)	14 (64)
Headache	3 (75)	4 (67)	5 (42)	12 (55)
Dysphonia	1 (25)	2 (33)	8 (67)	11 (50)
Fatigue	2 (50)	1 (17)	3 (25)	6 (27)
Nasopharyngitis	–	1 (17)	4 (33)	5 (23)
Arthralgia	–	2 (33)	2 (17)	4 (18)
Constipation	1 (25)	1 (17)	2 (17)	4 (18)
Epistaxis	1 (25)	1 (17)	2 (17)	4 (18)
Nausea	–	1 (17)	2 (17)	3 (14)
All G 3/4 events				
Hypertension	3 (75)	2 (33)	6 (50)	11 (56)
Headache	1 (25)	–	1 (8)	2 (9)
Pneumonia	–	1 (17)	1 (8)	2 (9)
Confusion	1 (25)	1 (17)	–	2 (9)
Pulmonary embolism	–	1 (17)	1 (8)	2 (9)
Fatigue	–	1 (17)	–	1 (5)
Deep vein thrombosis	1 (25)	–	–	1 (5)
Device-related infection	–	–	1 (8)	1 (5)
Meningitis associated with spinal fluid leak	–	1 (17)[a]	–	1 (5)
Sepsis	–	–	1 (8)	1 (5)
Tooth abscess	–	–	1 (8)	1 (5)
Urinary tract infection	–	–	1 (8)	1 (5)
Brain edema	–	–	1 (8)	1 (5)
Cerebral infarction	–	–	1 (8)[a]	1 (5)
Asthenia	–	–	1 (8)	1 (5)
Mucosal inflammation	–	–	1 (8)	1 (5)
Abdominal pain upper	–	–	1 (8)	1 (5)

[a]Indicates the 2 dose-limiting toxicities.
Abbreviations: AE, adverse event; G, grade; SAE, serious adverse event.

pulmonary embolism, confusion, and pneumonia occurred in more than one participant. All SAEs of hypertension and pulmonary embolism, meningitis associated with spinal fluid leak, upper abdominal pain, and cerebral infarction were considered to be study drug related. Four other SAEs were deemed possibly related but unexpected.

There were no apparent dose-related changes in vital signs, physical examinations, performance status, ECGs, or laboratory parameters. All lower extremity ultrasounds were normal at final visit.

Pharmacokinetics

Linear dose-dependent increases in peak and trough concentrations of RO5323441 were observed (Supplementary Table S1). The PK parameters of RO5323441 were estimated using a 2-compartment population PK model. The average effective half-life was 18.5 ± 8.0 days, and the mean apparent clearance was 0.19 ± 0.05 L/day. The mean estimated volume of distribution was 2.9 ± 0.6 L for the central compartment and 2.1 ± 1.5 L for the peripheral compartment. The mean area under the concentration-time curve over the dosing interval (AUC_{tau}) at steady state was 4884 ± 1142, 6075 ± 1019, and 12762 ± 3183 μg*day/mL for the 625, 1250, and 2500 mg dose levels, respectively. The linear dose-exposure relationship is shown in Supplementary Fig. S1). Bevacizumab serum exposures were similar across cohorts and were unaffected by the concomitant administration of RO5323441 (Supplementary Table S2).

Pharmacodynamics

Following initial treatment with single-agent bevacizumab (on day −14), DCE-MRI analysis revealed a large relative decrease from baseline (~60% by day −2) in initial area under the gadolinium concentration curve normalized with plasma input function (AUC_{BN}). AUC_{BN} is a composite parameter that reflects flow, permeability, and vascular volume.[23] This

effect (observed in all evaluated lesions in all participants) was maintained for the duration of combination therapy (Fig. 1B and C). A further decrease (day −2 to day 1) occurred for 2 participants after RO5323441 dosing. All participants also showed a marked decrease (∼70% overall) in DCE-MRI-derived fractional extracellular extravascular volume (v_e; Fig. 1D). DW-MRI revealed an overall smaller decrease (∼20%) in the water diffusion coefficient (ADC), and a reduction in ADC was observed in 4 of 6 participants (5/7 tumor lesions; Fig. 1E).

Baseline median plasma PlGF level (available for 21 participants) was 24.4 pg/mL (range, 14.1–31.3 pg/mL). In the expansion cohort, the median baseline PlGF level was 25.5 pg/mL (range, 20–31.3 pg/mL), which increased by ∼40% after a single dose of bevacizumab. In all cohorts, there was no apparent association between baseline PlGF levels and clinical response. The combined effect of bevacizumab plus RO5323441 on PlGF levels could not be evaluated due to assay interference caused by RO5323441.

Increases in VEGFA were observed following the administration of both combination therapy (cohorts 1–3) and bevacizumab monotherapy (cohort 4; Fig. 2A). Following prolonged combination treatment, the levels of VEGFA appeared to increase to a greater extent with the highest tested dose of RO5323441 (cohorts 3 and 4). Although results were variable, decreased levels of FLT4 occurred following the administration of both treatments (Fig. 2B).

Efficacy

Of the 22 treated participants, one (4.5%) participant had a complete response, 4 (18.2%) had a partial response, 11 (50%) had stable disease, and 6 (27.2%) had progressive disease (Supplementary Table S3 and Fig. 3A). Two participants experienced prolonged durable responses of 16 and 17 months after treatment with 1250 mg and 625 mg RO5323441, respectively. One participant had no on-treatment response assessments and was therefore considered to have progressive disease. These findings translate into an ORR of 22.7% and a DCR of 72.7%. No dose-dependent effect was observed.

Median PFS was 3.5 months (95% CI, 2.6–4.3 months; Fig. 3B) and median OS was 8.5 months (95% CI, 7.3–9.9 months; Fig. 3C). PFS-6 was 22.7%, and the Kaplan-Meier estimate for 6-month OS was 81.8%.

Corticosteroid use decreased in 6 (38%) of 16 participants who received corticosteroids at baseline (median dose reduction −50%; range, −20% to −80%).

Discussion

RO5323441 in combination with bevacizumab had acceptable tolerability in participants with recurrent glioblastoma. Two DLTs occurred, which led to participant withdrawal; however, the MTD for the combination was not reached. The highest RO5323441 dose tested was 2500 mg. The safety profile of RO5323441 was similar across dose groups, and no participant died while on the study. Hypertension was also the most frequent treatment-related AE (64% of participants) but was well managed with medication and did not lead to study discontinuations. The reason for the higher frequency of hypertension compared with previous studies with single-agent RO5323441[19] (5%) or single-agent bevacizumab[24] (30%–40%; 4%–11% G3/4) remains unclear. This may reflect the 30%–50% higher RO5323441 exposure seen in this study compared with that seen in patients with hepatocellular, ovarian, and colorectal cancer treated with equivalent RO5323441 doses as monotherapy/combination therapy (Roche, unpublished data). Conversely, bevacizumab exposure was not affected by coadministration with RO5323441. Dysphonia,

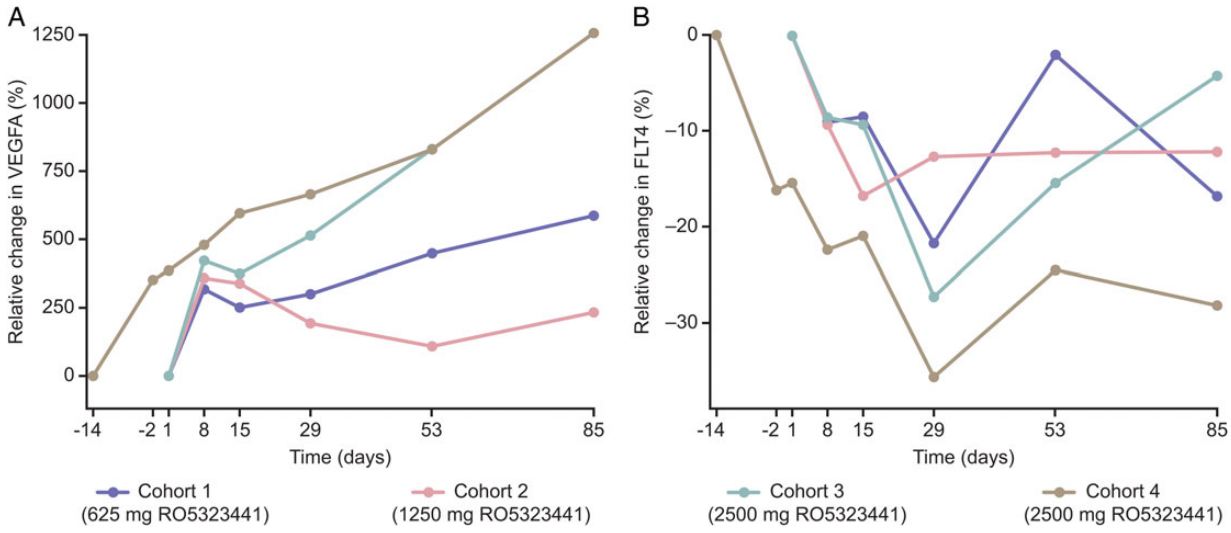

Fig. 2. Effect of antiangiogenic treatment on plasma levels of VEGFA (A) and FLT4 (B). All participants received bevacizumab 10 mg/kg combined with 625 mg (cohort 1), 1250 mg (cohort 2) or 2500 mg (cohorts 3 and 4) RO5323441 q2w. Combination dosing began on day 1 in cohorts 1–3. In cohort 4 (expansion cohort) participants received single-agent bevacizumab on day −14 and day 1, single-agent RO5323441 on day −2, with combination dosing beginning on day 15. Means of the relative change from baseline over time for each cohort are displayed. Abbreviations: FLT4, fms-related tyrosine kinase-4; VEGFA, vascular endothelial growth factor A.

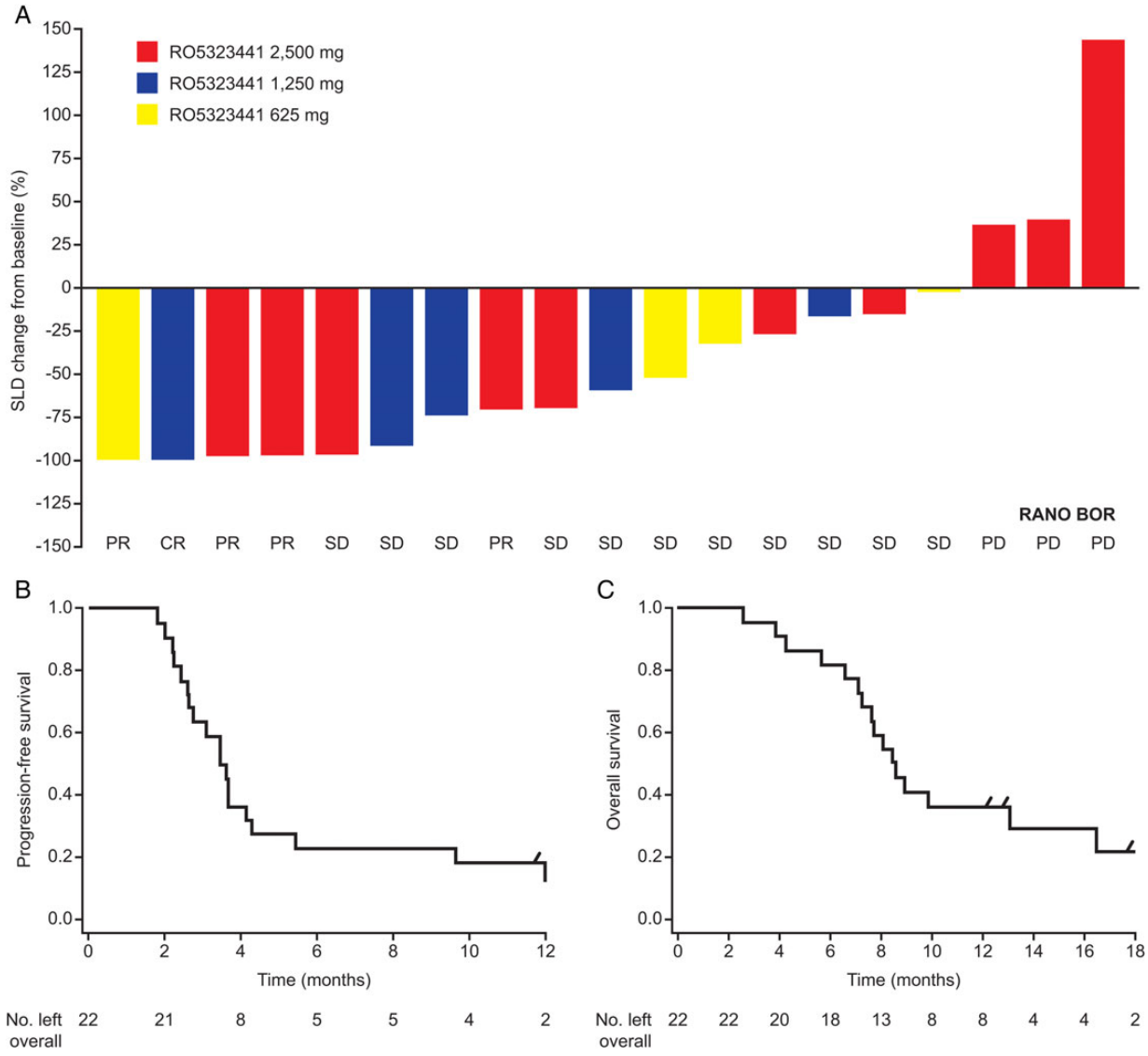

Fig. 3. Antitumor activity of RO5323441 and bevacizumab in glioblastoma patients. Waterfall plot showing maximum change in SLDs compared with baseline (A) and Kaplan-Meier curves of progression-free survival (B) and overall survival (C) for all patients. Figure 3A shows the data for 19 participants because 2 participants had no target lesions, and one had no post-treatment tumor assessments. Tick marks on Kaplan-Meier curves indicate censored data. Abbreviations: BOR, best overall response; CR, complete response; PD, progressive disease; PR, partial response; RANO, Revised Assessment in Neuro-Oncology; SD, stable disease; SLD, summed longest tumor diameter.

which is uncommon with single-agent bevacizumab[24] and single-agent RO5323441,[18,19] occurred frequently with combination therapy and may indicate a synergistic toxicity; however, all events were grade 1/2.

Consistent with the known antiangiogenic effects of bevacizumab,[25] VEGF inhibition was associated with a large decrease in DCE-MRI AUC_{BN}. Further reductions in AUC_{BN} occurred in 2 participants following subsequent administration of RO5323441, which may indicate an additive antiangiogenic effect. The reductions in DW-MRI ADC and DCE-MRI v_e reported here are comparable to previous results with other antiangiogenic treatments.[15] This suggests that the 2 agents may be capable of reducing vasogenic edema, a finding that has been previously reported with bevacizumab.[11,26] Bevacizumab therapy can also decrease (peri-)tumoral edema in participants with recurrent glioblastoma, thereby reducing the demand for corticosteroids.[27] This is consistent with the reduction in dexamethasone dose seen in 38% of participants who were receiving steroids at baseline in the current study.

Increases in plasma PlGF levels occur in patients with glioblastoma treated with anti-VEGF therapies[15,28] and may represent an escape mechanism to antiangiogenic therapy.[29] In this study, baseline plasma PlGF levels were comparable with previous results[15] and increased moderately following bevacizumab administration. The influence of RO5323441 on PlGF levels could not be assessed due to interference of

RO5323441with the assay. Across all cohorts, there was no apparent association between baseline PlGF levels and clinical response.

Bevacizumab therapy can increase levels of VEGF and decrease levels of VEGFRs.[30,31] PlGF has been proposed to stimulate angiogenesis by displacing VEGF from the "VEGFR-1 sink," thereby increasing the fraction of VEGFA available to activate VEGFR-2.[32] Hence, neutralizing PlGF by escalating doses of RO5523441 should decrease VEGFA levels by allowing increased binding of VEGF to VEGFR-1. However, apparently greater increase in VEGFA levels was seen in participants treated at the highest dose of RO5323441 (Fig. 2A), suggesting a (over-) compensatory feedback mechanism of VEGFA expressing tumor cells. Moreover, increasing doses of RO5523441 may also reduce the formation of PlGF/VEGF heterodimers,[33] which consequently increases VEGFA levels.

The ORR was 22.7% with no apparent differences between RO5323441 dose groups (no formal comparison of RO5323441 dose level and efficacy was conducted due to the low number of participants treated with the lower doses). Six-month PFS was 22.7%, and median OS was 8.5 months. These results are similar to previous findings with single-agent bevacizumab in recurrent glioblastoma,[10,11] including the recent BELOB[34] and CABARET[35] studies (16%–24% PFS-6). While dual inhibition of VEGF and PlGF did not increase the ORR compared with previous studies of single-agent bevacizumab, 2 participants did experience durable responses of 16 and 17 months, respectively. The value of PlGF as a therapeutic target in cancer remains undetermined.[36,37] Results with aflibercept, which binds both VEGFR and PlGF, have also been disappointing in patients with glioblastoma (PFS-6 was 7.7%), despite the encouraging activity of this agent in other cancers.[38]

In summary, the safety and tolerability of multiple-dose RO5323441 and bevacizumab were acceptable and manageable, and the MTD for the combination therapy was not determined. While our study was not designed to test the efficacy of RO5323441, the data suggest that dual anti-VEGF and anti-PlGF inhibition with bevacizumab and RO5323441 in recurrent glioblastoma offers no therapeutic advantage over that which can be achieved with bevacizumab alone. The clinical development of RO5323441 has been terminated by the sponsor following an overall portfolio review.

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