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A randomised, double-blind, placebo-controlled phase 2 study of trebananib (AMG 386) in combination with FOLFIRI in patients with previously treated metastatic colorectal carcinoma

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Background: This phase 2 study evaluated trebananib (AMG 386), an investigational peptide-Fc fusion protein that neutralises the interaction between angiopoietins-1/2 and the Tie2 receptor, plus FOLFIRI as second-line treatment for patients with metastatic colorectal cancer.

Methods: Patients had adenocarcinoma of the colon or rectum with progression within 6 months of receiving only one prior fluoropyrimidine/oxaliplatin-based chemotherapy regimen for metastatic disease. All patients received FOLFIRI and were randomised 2 : 1 to also receive intravenous trebananib 10 mg kg^{-1} once weekly (QW) (Arm A) or placebo QW (Arm B). The primary end point was investigator-assessed progression-free survival (PFS).

Results: One hundred and forty-four patients were randomised (Arms A/B, $n = 95/49$). Median PFS in Arms A and B was 3.5 and 5.2 months (hazard ratio (HR) 1.23; 95% CI, 0.81–1.86; $P = 0.33$) and median overall survival (OS) was 11.9 and 8.8 months, respectively (HR 0.90; 95% CI; 0.53–1.54; $P = 0.70$). Objective response rate (ORR) was 14% and 0% in Arms A and B, respectively. Incidence of grade $\geqslant 3$ adverse events was similar between treatment arms (Arm A, 61%; Arm B, 65%) and included pulmonary embolism (1%/4%), deep vein thrombosis (5%/2%), and hypertension (1%/0%).

Conclusion: Administration of trebananib plus FOLFIRI did not prolong PFS compared with placebo plus FOLFIRI. Toxicities were manageable and consistent with those known for FOLFIRI and trebananib.

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Current first- and second-line therapies for metastatic colorectal cancer (mCRC) include a variety of different oxaliplatin- and irinotecan-based chemotherapy regimens (Van Cutsem *et al*, 2010). Improved outcomes have been demonstrated with chemotherapy combined with therapies targeting the epidermal growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF) (Van Cutsem *et al*, 2010). However, overall survival (OS) times remain relatively short and investigation of alternative treatment strategies is warranted.

Angiogenesis is a complex process that has an important role in tumour development, growth, and metastasis (Carmeliet and Jain, 2011). The VEGF pathway and the angiopoietin-Tie2 receptor axis have distinct roles in the regulation of pathologic angiogenesis (Huang *et al*, 2010). Evidence suggests that the angiopoietins may be implicated in colorectal cancer. Elevated serum angiopoietin-2 has been reported in patients with colorectal cancer compared with healthy controls (Goede *et al*, 2010), and increased serum angiopoietin-2 has been associated with poorer survival outcomes (Volkova *et al*, 2011). Furthermore, higher tumour angiopoietin-2 expression has been associated with lymph node metastasis, venous invasion, and microvascular density (Chung *et al*, 2006). Preclinical evidence suggests there may be interactions between the angiopoietin axis and other signalling pathways, including the EGFR pathway (Fujiyama *et al*, 2001) that could contribute to tumour angiogenesis. Potentially, inhibiting angiogenesis via blockade of the angiopoietin axis may represent a novel treatment approach for colorectal cancer.

Trebananib (formerly known as AMG 386) is an investigational, intravenously administered peptide-Fc fusion protein that neutralises the interaction between angiopoietin-1 and angiopoietin-2 and the Tie2 receptor. In a Colo205 colorectal cancer tumour xenograft model, blocking the angiopoietin-2/Tie2 interaction inhibited tumour growth (Oliner *et al*, 2004). Importantly, administration of peptibodies targeting angiopoietin-1 or angiopoietin-2 was less effective in inhibiting Colo205 xenograft growth than dual inhibition of angiopoietin-1 and angiopoietin-2 (either by combined administration of anti-angiopoietin-1– and anti-angiopoietin-2–peptibodies or by administration of trebananib) (Coxon *et al*, 2010). Trebananib has shown encouraging antitumour activity and exhibited a specific toxicity profile when administered as monotherapy (Herbst *et al*, 2009) or in combination with various chemotherapy regimens (Mita *et al*, 2010), including weekly paclitaxel in patients with recurrent ovarian cancer (Karlan *et al*, 2012). The primary objective of our study was to estimate the treatment effect (as assessed by progression-free survival (PFS)) of second-line trebananib plus FOLFIRI *vs* placebo plus FOLFIRI in patients with mCRC.

MATERIALS AND METHODS

Patients. Eligible patients ($\geqslant$18 years) had histologically confirmed, metastatic adenocarcinoma of the colon or rectum, had received only one prior fluoropyrimidine- and oxaliplatin-based chemotherapy regimen for metastatic disease, had measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (Therasse *et al*, 2000), and had radiographically documented disease progression per RECIST during or within 6 months of their last chemotherapy dose. Other eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status $\leqslant$1; life expectancy $\geqslant$3 months; and adequate haematologic, renal, hepatic, and haemostatic function. Key exclusion criteria were arterial or deep venous thromboembolism within 12 months of randomisation; clinically significant bleeding within 6 months; clinically significant cardiovascular disease within 12 months; and nonhealing wound, ulcer, or fracture; radiotherapy within 14 days (patients must have recovered from all radiotherapy-related toxicities); prior therapy with angiopoietin-Tie2 axis inhibitors; and prior irinotecan therapy. Prior treatment with anticancer agents other than irinotecan was allowed with a sufficient washout period before randomisation (30 days for proteins/antibodies (including bevacizumab) and 21 days for other agents) and prior adjuvant chemotherapy (in addition to first-line therapy) was allowed if it preceded the onset of metastatic disease. All patients provided written informed consent; study procedures were approved by independent ethics committees/institutional review boards.

Study design and treatment. This randomised, double-blind, placebo-controlled phase 2 study was conducted at 38 international sites. Using an interactive voice response system, patients were randomly assigned 2:1 to receive (Arm A) intravenous (IV) trebananib 10 mg kg^{-1} once weekly (QW) plus FOLFIRI (irinotecan 180 mg m^{-2} IV plus leucovorin 400 mg m^{-2} IV plus 5-FU 400 mg m^{-2} IV bolus followed by 2400 mg m^{-2} continuous IV infusion) once every 2 weeks (Q2W), or (Arm B) placebo QW plus FOLFIRI Q2W. Randomisation was stratified by ECOG status (0 *vs* 1). Patients, investigators, and study staff were blinded to treatment assignments. Treatment continued until disease progression, unacceptable toxicity, or withdrawal of consent. Dose modifications were not permitted for trebananib or placebo. Treatment was permanently discontinued if withheld for $>$28 days or $>$2 times because of treatment-related toxicity or in the event of the following toxicities: central nervous system haemorrhage (any grade), haemorrhage (grade $\geqslant$3), grade 4 symptomatic venous thromboembolic event, or arterial thrombosis (any grade).

The primary end point was PFS. Secondary end points included objective response rate (ORR) per RECIST (Therasse *et al*, 2000), duration of response, time to response, OS, incidence of adverse events (AEs), incidence of anti-trebananib antibodies, and assessment of trebananib pharmacokinetics. Exploratory end points included assessment of pharmacodynamic biomarkers. Furthermore, PFS was also evaluated by *KRAS* mutational status.

Efficacy assessments. Radiologic tumour measurement (computed tomography/magnetic resonance imaging) was performed at baseline and every 8 $\pm$ 1 weeks thereafter. Responses were assessed according to RECIST version 1.0 (Therasse *et al*, 2000) by investigators and confirmed $\geqslant$28 days after the initial criteria for response were met. Patients who discontinued treatment without progressive disease or withdrew consent continued scheduled response assessments until disease progression or initiation of new therapy. For patients discontinuing treatment because of progression or unacceptable toxicity, long-term follow-up is being conducted every 3 months through 30 months from the date the last patient was randomised.

Toxicity assessments. Adverse events were recorded and graded for severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. A safety follow-up visit occurred 30–37 days after a patient discontinued the study for any reason. Serum samples for measurement of human anti-trebananib binding and neutralising antibodies (evaluated as described previously) (Herbst *et al*, 2009) were collected predose on day 1 of weeks 1, 5, and 9; every 16 weeks thereafter; and at the safety follow-up visit.

Pharmacokinetics and biomarkers. Methods for pharmacokinetic analysis of trebananib, 5-FU, SN-38, and irinotecan and analysis of the biomarkers angiopoietin-1, angiopoietin-2, placental growth factor (PLGF), soluble vascular cell adhesion molecule-1 (sVCAM-1), VEGF-A, soluble VEGF receptors 1 and 2, and soluble Kit are described in the Supplementary Material.

Statistical analysis. In this phase 2 study, the planned enrolment of 138 patients (Arm A, $n=92$; Arm B, $n=46$) was intended to generate treatment effect estimates of trebananib plus FOLFIRI *vs* placebo plus FOLFIRI. The primary analysis was planned for when 100 PFS events had occurred. The primary statistical analysis was estimation of the hazard ratio (HR) of PFS. With a hypothesised HR of 0.69, this allowed estimation of the HR for PFS with a two-sided 80% CI with a maximum half-width of 0.22. Efficacy end points were analysed for the intent-to-treat population (all randomised patients). The safety analysis set included all patients who received $\geqslant 1$ dose of study treatment.

A Cox regression model stratified by ECOG performance status was used to estimate HRs and two-sided 80% CIs and 95% CIs for PFS (time from randomisation to disease progression per RECIST or death) and 95% CIs for OS (time from randomisation to death). Kaplan–Meier estimates of median (95% CI) PFS, OS, time to response, and duration of response were also derived (Brookmeyer and Crowley, 1982). Exact binomial two-sided 95% CIs were generated for ORR for both treatment arms. The 95% CI for the difference in ORR between treatment arms was calculated using Wilson's score method with continuity correction (Newcombe, 1998). Analyses of PFS, OS, and ORR by *KRAS* mutation status were performed for each *KRAS* subgroup using similar methods.

RESULTS

Patients. Between December 2008 and May 2010, 144 patients were randomised (Arm A, $n=95$; Arm B, $n=49$). All but one patient in Arm A received $\geqslant 1$ dose of trebananib/placebo. Baseline demographics and clinical characteristics were generally balanced in both treatment arms (Table 1). Twenty-one patients in Arm A and 9 in Arm B had received antiangiogenic agents before the study, including 20 and 8 patients, respectively, who had received bevacizumab. The proportion of patients with wild-type, mutant, or unknown *KRAS* status was 49%, 36%, and 15%, respectively, in Arm A, and 59%, 29%, and 12%, respectively, in Arm B. At the time of this primary analysis, 15 patients in Arm A and 12 in Arm B continued to receive study treatment; reasons for discontinuation are shown in Figure 1. Patients in Arm A received a median (range) of 9 (1–57) trebananib infusions; patients in Arm B received 16 (2–55) placebo infusions. A median of 6 and 8 cycles of FOLFIRI were administered in Arms A and B, respectively. Median follow-up time was 27.3 weeks for Arm A and 24.4 weeks for Arm B. Forty-six per cent of patients in Arm A and 38% of patients in Arm B received anticancer treatment after progression. More patients in Arm A than Arm B received chemotherapy plus anti-EGFR (12% *vs* 3%) or anti-VEGF (5% *vs* 0%) therapy post-progression.

PFS and OS. At the time of this analysis, 72 (76%) and 35 (71%) patients in Arms A and B, respectively, had had disease progression or died. The HR for PFS was 1.23 (95% CI, 0.81–1.86; $P=0.33$) and median PFS was 3.5 months in Arm A *vs* 5.2 months in Arm B (Table 2; Figure 2). Overall survival data were not mature at the time of this primary analysis: 40% of patients in Arm A and 43% of patients in Arm B had died. Median estimated OS in Arms A and B was 11.9 and 8.8 months, respectively (HR, 0.90; 95% CI, 0.53–1.54; $P=0.70$; Table 2).

ORR. The confirmed ORR was 14% in Arm A (including two complete responses) and 0% in Arm B (Table 2). The median duration of response for patients in Arm A was 27.1 weeks, and the mean time to response was 12.9 weeks. The proportion of patients with reductions in tumour size from baseline was 64% and 59% in Arms A and B, respectively.

Outcomes by *KRAS* status. Among patients with wild-type *KRAS* tumours ($n=76$), median PFS was 5.2 months in Arm A and 4.5 months in Arm B (HR, 0.96; 95% CI, 0.56–1.67; $P=0.89$). For those with mutant *KRAS* tumours ($n=48$), median PFS was 2.8 months in Arm A and 5.5 months in Arm B (HR, 2.10; 95% CI, 0.84–5.25; $P=0.12$). Corresponding median OS times were 11.9 months in Arm A and 12.1 months in Arm B for patients with wild-type *KRAS* (HR, 0.86; 95% CI, 0.40–1.85; $P=0.70$) and 9.6 and 8.8 months, respectively, for those with mutant *KRAS* (HR, 1.04; 95% CI, 0.39–2.77; $P=0.94$). The ORR for Arm A patients with wild-type *KRAS* was 17.5% *vs* 10.0% for those with mutant *KRAS*.

Toxicity. The most frequently occurring AEs in both arms were diarrhoea, nausea, and neutropenia (Table 3). Generally, the incidence of AEs of any grade was similar across the treatment arms. Exceptions included peripheral oedema, which occurred more often in Arm A (20% *vs* 4% in Arm B; no grade $\geqslant 3$), and neutropenia, vomiting, and anaemia, which were more frequent in Arm B (Table 3). Both treatment arms also had a similar incidence of grade $\geqslant 3$ AEs (62% in Arm A *vs* 65% in Arm B) and serious AEs (28% *vs* 33%), and 12% of patients in each arm discontinued treatment or the study because of AEs. There were six (6%) fatal events in Arm A and three (6%) in Arm B. Of these, metastatic colon/colorectal cancer (Arm A, $n=2$) and cardiorespiratory arrest (Arm B, $n=2$) occurred in >1 patient. Other fatal AEs in Arm A were diarrhoea, suicide, pulmonary oedema, and acute myocardial infarction; one patient in Arm B had a fatal AE reported as 'disability'. None of the fatal AEs were considered by study investigators to be related to study treatment.

The incidence of AEs identified as being of specific interest before the study was initiated (including arterial and venous thromboembolic events, hypertension, and perforations) was generally similar across both treatment arms (Table 4); however, some AEs warrant special mention. There was one gastrointestinal perforation (grade 3 abdominal abscess) and one grade 5 pulmonary oedema (both in Arm A). Additionally, one patient in Arm A had grade 5 acute myocardial infarction, one patient had grade 4 pulmonary embolism, and one patient had grade 4 cerebral venous thrombosis. In Arm B, one patient had grade 4 arterial thrombosis and two patients had grade 4 pulmonary embolism.

Pharmacokinetics. Median (per cent coefficient of variation (CV%)) trebananib C_{max} (221 $\mu g\,ml^{-1}$ (69.8%); $n=64$) and C_{min} (15.6 $\mu g\,ml^{-1}$ (56.2%); $n=74$) following coadministration with FOLFIRI at week 5 were similar to those reported in the first-in-human phase 1 monotherapy study (236 $\mu g\,ml^{-1}$ and 13.7 $\mu g\,ml^{-1}$, respectively) (Herbst *et al*, 2009). Intensive trebananib pharmacokinetic analysis was performed for seven patients (Figure 3A). Among these patients, mean (CV%) steady-state clearance for trebananib was 1.76 $ml\,h^{-1}\,kg^{-1}$ (31.0%). At week 5, median (CV%) plasma irinotecan C_{max} was similar in Arms A and B (1800 $ng\,ml^{-1}$ (54.7%) and 1970 $ng\,ml^{-1}$ (37.9%), respectively). Week 5 median (CV%) plasma SN-38 C_{max} values were lower in Arm A than Arm B (22.4 $ng\,ml^{-1}$ (61.5%) *vs* 31.6 $ng\,ml^{-1}$ (62.3%)). However, variability within each arm was high and the difference was not statistically significant. Median (CV%) steady-state plasma concentrations (C_{ss}) for 5-FU were also lower in Arm A than Arm B at both week 1 (542 $ng\,ml^{-1}$ (345%) *vs* 1310 $ng\,ml^{-1}$ (306%)) and week 5 (347 $ng\,ml^{-1}$ (328%) *vs* 560 $ng\,ml^{-1}$ (151%)). Again, variability within each arm was high and the difference was not statistically significant. Median C_{ss} in Arm B at week 1 was higher for women than for men (Figure 3).

Anti-trebananib antibodies. Pre-existing nonneutralising anti-trebananib antibodies were detected in 3 out of 90 patients in Arm A, and postbaseline nonneutralising anti-trebananib antibodies

Table 1. Baseline demographics and clinical characteristics

	Arm A Trebananib 10 mg kg^{-1} QW + FOLFIRI (*n* = 95)	Arm B Placebo + FOLFIRI (*n* = 49)
Men, *n* (%)	60 (63)	24 (49)
Median (range) age, years	56 (23–79)	55 (29–79)
Region, *n* (%)		
Asia	20 (21)	11 (22)
Australia	25 (26)	7 (14)
Europe	46 (48)	31 (63)
North America	4 (4)	0 (0)
Race/ethnicity, *n* (%)		
White	73 (77)	37 (76)
Asian	20 (21)	12 (24)
Black	2 (2)	0 (0)
Primary tumour type, *n* (%)		
Colon	48 (51)	25 (51)
Rectal	47 (49)	24 (49)
ECOG performance status, *n* (%)		
0	50 (53)	22 (45)
1	45 (47)	27 (55)
Median (range) time since primary diagnosis, months	11.7 (3–103)	13.0 (5–107)
Disease stage at screening, *n* (%)		
IV	95 (100)	49 (100)
Metastatic sites, *n* (%)		
1	26 (27)	6 (12)
2	29 (31)	19 (39)
3	23 (24)	12 (24)
⩾4	17 (18)	12 (24)
Liver metastases, *n* (%)	70 (74)	33 (67)
Prior adjuvant chemotherapy, *n* (%)	22 (23)	11 (22)
Prior antiangiogenic therapy, *n* (%)	**21 (22)**	**9 (18)**
Bevacizumab	20 (21)	8 (16)
Antiangiogenic tyrosine kinase inhibitor	3 (3)	2 (4)
***KRAS* status,[a] *n* (%)**		
Mutant	34 (36)	14 (29)
Wild type	47 (49)	29 (59)
Unknown[b]	14 (15)	6 (12)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; NA = not available; QW = once weekly.
[a]Mutations in *KRAS* codons 12 and 13 were assessed using the RUO KR-04 *KRAS* Mutation Test Kit (DxS Ltd., Manchester, UK).
[b]Includes patients for whom DNA of insufficient quantity or quality was obtained or for whom no tumour specimen was available.

developed in 1 out of 85 patients. No patient had anti-trebananib neutralising antibodies.

Biomarkers. Of the eight biomarkers tested in this study two showed a notable pharmacodynamic response. After initiation of treatment, serum PLGF increased above baseline in both Arms A and B; this increase was greater in Arm A from week 1 to week 13 (Supplementary Figure 1). Similarly, serum sVCAM-1 was elevated above baseline in both treatment arms throughout the study period, with a greater increase in Arm A than in Arm B (Supplementary Figure 2). For both PLGF and sVCAM-1, greatest increases above baseline in Arm A were measured at week 1 and 5 postdose assessments. There were limited or no changes from baseline in other biomarkers and no associations between any of the tested biomarkers and clinical outcomes (data not shown). Angiopoietin-1 and -2 could only be measured at baseline due to assay interference from trebananib present in the serum samples. Further analysis showed no association between baseline levels of these two markers and outcomes, specifically PFS (data not shown).

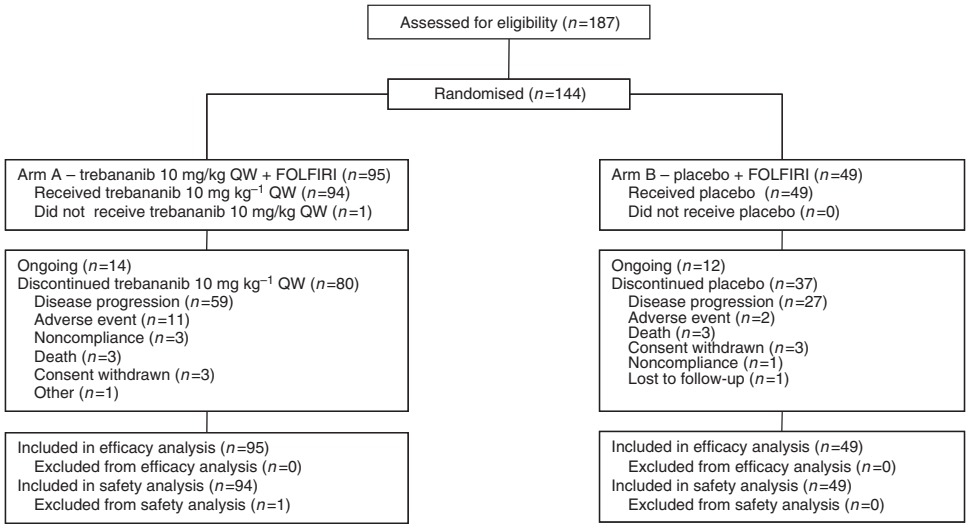

Figure 1. Disposition of study patients. Noncompliance includes patients who did not comply with study drug administration, visit schedule, or other protocol requirement(s). QW = once weekly.

Table 2. Efficacy

	Arm A **Trebananib 10 mg kg^{-1} QW + FOLFIRI (*n* = 95)**	**Arm B** **Placebo + FOLFIRI (*n* = 49)**
PFS		
Median (95% CI) Kaplan–Meier PFS time, months	3.5 (2.5–5.3)	5.2 (3.7–5.5)
Cox regression model		
Arm A *vs* Arm B, HR (95% CI)	1.23 (0.81–1.86)	
80% CI	0.94–1.61	
P-value	0.33	
P-value, stratified log-rank test	0.32	
OS		
Median (95% CI) Kaplan–Meier OS time, months	11.9 (9.2–14.8)	8.8 (7.1–NE)
Cox regression model		
Arm A *vs* Arm B, HR (95% CI)	0.90 (0.53–1.54)	
P-value	0.70	
P-value, stratified log-rank test	0.71	
Objective response		
Best confirmed response, *n* (%)		
Confirmed CR	2 (2)	0 (0)
Confirmed PR	10 (12)	0 (0)
Stable disease	38 (45)	31 (69)
Stable disease >16 weeks	19 (23)	21 (47)
Progressive disease	28 (33)	10 (22)
Unevaluable[a]	0 (0)	1 (2)
Not done[b]	6 (7)	3 (7)
Confirmed objective response rate (CR + PR), % (95% CI)	14 (8–24)	0 (0–8)
Mean (95% CI) time to response, week	12.9 (8.9–16.9)	—
Median (95% CI) duration of response, week	27.1 (24.3–36.3)	—

Abbreviations: CI = confidence interval; CR = complete response; HR = hazard ratio; NE = not estimable; OS = overall survival; PFS = progression-free survival; PR = partial response; QW = once weekly.

[a]Patients with response assessments of CR, PR or SD before the first scheduled response assessment who did not undergo a subsequent response assessment.

[b]Imaging was not performed at the scheduled tumour assessment.

DISCUSSION

In this phase 2 study, the combination of trebananib plus FOLFIRI had acceptable toxicity but did not prolong PFS compared with placebo plus FOLFIRI. In contrast, ORR appeared to favour patients in Arm A *vs* Arm B, although the proportion of patients with reductions in tumour size from baseline was similar. There were no apparent imbalances in prognostic/predictive factors that might have influenced the PFS results, and there is no clear explanation for the lack of correlation between PFS and ORR. Trebananib pharmacokinetic parameters were consistent with those reported in previous studies (Herbst *et al*, 2009; Mita *et al*, 2010; Karlan *et al*, 2012), but suggested reduced exposure to SN-38 (an irinotecan metabolite) and 5-FU among patients in Arm A. However, because the data were highly variable any contribution of this finding to the efficacy outcomes is difficult to assess.

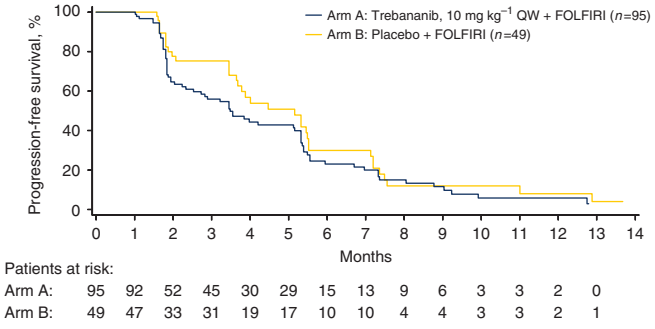

Figure 2. Progression-free survival among patients randomised to trebananib 10 mg kg^{-1} QW plus FOLFIRI or placebo plus FOLFIRI. QW = once weekly.

Table 4. Patient incidence of adverse events of specific interest

Adverse events of specific interest,[a] *n* (%)	Arm A Trebananib 10 mg kg^{-1} QW + FOLFIRI (*n* = 95)	Arm B Placebo + FOLFIRI (*n* = 49)
Arterial thromboembolic events	2 (2)	1 (2)
Grade 3	1 (1)	0 (0)
Grade 4	0 (0)	1 (2)
Grade 5	1 (1)	0 (0)
Venous thromboembolic events	9 (10)	4 (8)
Grade 3	5 (5)	1 (2)
Grade 4	2 (2)	2 (4)
Pulmonary oedema	1 (1)	0 (0)
Grade 5	1 (1)	0 (0)
Gastrointestinal perforation events	1 (1)	0 (0)
Grade 3	1 (1)	0 (0)
Haemorrhagic events	5 (5)	3 (6)
Grade 3	1 (1)	0 (0)
Grade 4	1 (1)	0 (0)
Hypertension	3 (3)	2 (4)
Grade 3	1 (1)	0 (0)
Proteinuria	1 (1)	0 (0)
Hypokalemia	3 (3)	0 (0)
Grade 3	1 (1)	0 (0)

Abbreviation: QW = once weekly.
[a]Unless otherwise indicated, all adverse events of interest were grade ⩽2.

Table 3. Patient incidence of adverse events

	Arm A Trebananib 10 mg kg^{-1} QW + FOLFIRI (*n* = 94)		Arm B Placebo + FOLFIRI (*n* = 49)	
Patients with any adverse event, *n* (%)	91 (97)		48 (98)	
Grade 3	41 (44)		19 (39)	
Grade 4	11 (12)		10 (20)	
Grade 5	6 (6)		3 (6)	
Adverse events occurring in ⩾10% of patients in either treatment arm, *n* (%)	All Grades	Grade ⩾3	All grades	Grade ⩾3
Diarrhoea	44 (47)	4 (4)	20 (41)	0 (0)
Nausea	41 (44)	0 (0)	18 (37)	1 (2)
Neutropenia	39 (41)	29 (31)	28 (57)	20 (41)
Asthenia	29 (31)	5 (5)	16 (33)	2 (4)
Decreased appetite	26 (28)	2 (2)	8 (16)	0 (0)
Alopecia	24 (26)	0 (0)	18 (37)	0 (0)
Fatigue	23 (24)	2 (2)	9 (18)	2 (4)
Constipation	20 (21)	0 (0)	9 (18)	1 (2)
Peripheral oedema	19 (20)	0 (0)	2 (4)	0 (0)
Vomiting	16 (17)	0 (0)	19 (39)	3 (6)
Abdominal pain	13 (14)	1 (1)	5 (10)	3 (6)
Pyrexia	13 (14)	2 (2)	4 (8)	0 (0)
Leucopenia	12 (13)	6 (6)	6 (12)	3 (6)
Stomatitis	12 (13)	2 (2)	4 (8)	0 (0)
Cough	7 (7)	0 (0)	7 (14)	0 (0)
Anaemia	6 (6)	2 (2)	12 (24)	3 (6)

Abbreviation: QW = once weekly.

A

	C_{max} (μg ml^{-1})	$AUC_{0\text{-tau}}$ (mg•h ml^{-1})	$t_{1/2,z}$ (h)	CL (ml h^{-1} kg^{-1})	C_{min} (μg ml^{-1})
N	7	7	7	7	7
Mean	160	6.07	88.9	1.76	12.8
SD	34.9	1.58	14.4	0.548	3.78
Min	110	3.51	68.5	1.20	5.39
Median	174	6.09	85.8	1.63	13.3
Max	198	8.35	108	2.85	17.1
CV%	21.8	26.0	16.2	31.0	29.5
Geometric mean	157	5.87	87.9	1.70	12.2

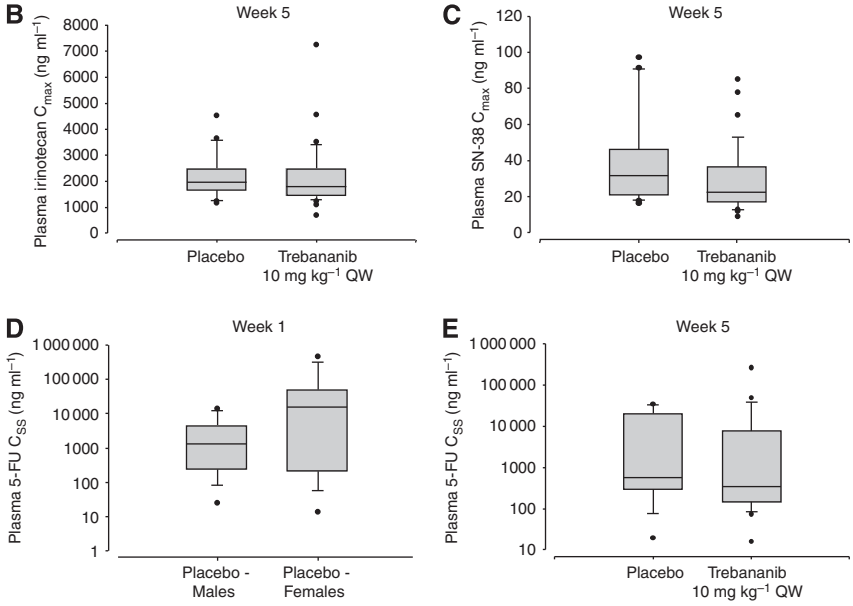

Figure 3. (**A**) Descriptive statistics for the pharmacokinetics of trebananib at week 5 among patients who received trebananib 10 mg kg^{-1} QW plus FOLFIRI (intensive pharmacokinetic analysis subset). (**B**) C_{max} at week 5 of irinotecan among patients who received trebananib 10 mg kg^{-1} QW plus FOLFIRI or placebo plus FOLFIRI. (**C**) C_{max} at week 5 of SN-38 among patients who received trebananib 10 mg kg^{-1} QW plus FOLFIRI or placebo plus FOLFIRI. (**D**) Baseline 5-FU C_{ss} at week 1 among patients who received placebo plus FOLFIRI by patient sex. (**E**) C_{ss} of 5-FU at week 5 among patients who received trebananib 10 mg kg^{-1} QW plus FOLFIRI or placebo plus FOLFIRI. C_{max} = maximum observed concentration; C_{ss} = concentration at steady state; CV = coefficient of variation; QW = once weekly.

PFS among patients in Arm B was longer than that reported for patients in other studies who received FOLFIRI following failure of a regimen containing 5-FU and/or oxaliplatin (2.5–4.7 months) (Tournigand *et al*, 2004; Peeters *et al*, 2010; Van Cutsem *et al*, 2011). This is somewhat surprising given that the eligibility criteria required that patients had progressed within 6 months of their most recent chemotherapy dose, which would have been expected to yield a population with relatively poor prognosis. In contrast, no patients in Arm B had an objective response, whereas previous studies have reported ORRs of 4% to 11% for patients receiving FOLFIRI following failure of 5-FU and/or oxaliplatin (Tournigand *et al*, 2004; Peeters *et al*, 2010; Van Cutsem *et al*, 2011). Notably, the estimated ORR in Arm A (14%) was not only higher than the ORR in Arm B but also higher than the historical range.

The incidence of AEs, grade $\geqslant$3 AEs, serious AEs, and AEs leading to discontinuation were similar for both treatment arms. The nature and incidence rate of toxicities in the trebananib arm were consistent with those reported in previous studies of trebananib administered as monotherapy (Herbst *et al*, 2009) or when combined with chemotherapy (Mita *et al*, 2010; Karlan *et al*, 2012); no new toxicity signals were identified. Trebananib had a specific toxicity profile. Peripheral oedema (no grade $\geqslant$3), which occurred more frequently in Arm A than in Arm B, appears to be a toxicity specific to trebananib treatment and has been reported in previous studies (Herbst *et al*, 2009; Mita *et al*, 2010; Karlan *et al*, 2012). Adverse events such as hypertension, haemorrhage, and thromboembolic events did not occur with greater incidence in Arm A than in Arm B. These AEs are of interest because they have been reported in studies of patients with mCRC receiving 5-FU–based chemotherapy plus VEGF pathway inhibitors (Hurwitz *et al*, 2004; Giantonio *et al*, 2007; Saltz *et al*, 2008; Van Cutsem *et al*, 2011). A distinct toxicity profile for trebananib is consistent with its mechanism of action of blocking the angiopoietin/Tie2 receptor pathway, separate from the VEGF cascade.

Trebananib exposure when coadministered with FOLFIRI was similar to that reported for trebananib 10 mg kg^{-1} administered as monotherapy (Herbst *et al*, 2009) or in combination with various chemotherapy regimens (Mita *et al*, 2010; Karlan *et al*, 2012). Pharmacokinetic parameters for irinotecan were comparable with and without trebananib administration. SN-38 and 5-FU exposures were lower in Arm A than Arm B; however, the data must be interpreted with caution considering the high pharmacokinetic variability. Given that trebananib is a peptibody and that 5-FU is metabolised by dihydropyrimidine dehydrogenase (DPD) (van Kuilenburg, 2004) and irinotecan undergoes glucuronidation by UGT1A1 (Gupta *et al*, 1994; Rouits *et al*, 2004), pharmacokinetic interactions were not anticipated. The data indicated higher plasma 5-FU concentrations in women *vs* men, which is consistent with previous studies showing that women generally have lower DPD expression, and thus metabolise 5-FU more slowly than men (Milano *et al*, 1992; Milano and McLeod, 2000; Yamashita *et al*, 2002; Kubota, 2003). The studies' findings might also explain the lower plasma 5-FU concentrations measured in Arm A, compared with Arm B, because more male patients were randomised to that arm.

There has been interest in the use of predictive biomarkers to identify patients with mCRC most likely to derive benefit from specific targeted therapies (Deschoolmeester *et al*, 2010). We tested

a panel of eight biomarkers in our study. Given the interdependent nature of angiogenic signalling pathways, the panel included molecules from both the angiopoietin/Tie2 axis and the VEGF pathway as well as molecules that are known to be involved in vascular remodelling (sVCAM), a consequence of angiopoietin signalling. Increases in serum levels of PLGF and sVCAM-1 occurred in both treatment arms; however, there was evidence of an additive effect for trebananib compared with placebo. Some research suggests that PLGF and sVCAM-1 have important roles in the development and progression of colorectal cancer (Velikova *et al*, 1998; Wei *et al*, 2005). We hypothesise that the observed changes in PLGF and sVCAM-1 reflect a response of the vasculature to trebananib. Both molecules have been proposed to be prognostic markers in various tumour types, including colorectal cancer (Silva *et al*, 2006; Okugawa *et al*, 2008; Willett *et al*, 2009; Bass *et al*, 2010). We tested for associations between changes in PLGF and sVCAM-1 and efficacy outcomes; however, none were identified in this study. Angiopoietin-1 and -2 were measured at baseline in each treatment arm but no association with PFS or other outcomes was found. Similarly, there was no evidence that *KRAS* status influenced outcomes. Additional work aimed at identifying a biomarker for trebananib is currently ongoing. Other molecules that could be tested may include the Tie2 receptor, platelet-derived growth factor, Notch, and molecules involved in vascular remodelling (e.g., intercellular adhesion molecule (ICAM)).

The chief limitation of this study was the relatively small number of patients enroled. Furthermore, evaluation of a higher dose of trebananib could have been of interest. A phase 1 study of trebananib in patients with solid tumours examined doses ranging from 0.3 mg kg^{-1} QW to 30 mg kg^{-1} QW. Although a maximum tolerated dose was not reached, pharmacokinetic data suggested that doses of 3–10 mg kg^{-1} would provide sufficient exposure to achieve antitumour activity (Herbst *et al*, 2009). In subsequent studies, trebananib doses up to 10 mg kg^{-1} QW in combination with several chemotherapy and targeted therapy regimens were evaluated (Mita *et al*, 2010; Eatock *et al*, 2012; Karlan *et al*, 2012; Rini *et al*, 2012). However, data from the phase 2 study of trebananib plus weekly paclitaxel for the treatment of recurrent ovarian cancer indicated a dose-response relationship (Karlan *et al*, 2012), and an exposure-response analysis of the results suggested that greater improvements in PFS might be achieved in that setting by administering trebananib at concentrations greater than 10 mg kg^{-1} (Lu *et al*, 2011). Given these data, assessment of trebananib in the present study at a dose higher than 10 mg kg^{-1} QW might have yielded different results. Three ongoing phase 3 trials in ovarian cancer are evaluating trebananib 15 mg kg^{-1} QW in combination with chemotherapy (NCT01204749, NCT01493505, and NCT01281254). Finally, the OS results are not yet mature and there were imbalances in post-progression therapy between the arms. Consequently, these data must be interpreted with caution.

In summary, administration of trebananib plus FOLFIRI in this estimation study did not prolong PFS compared with placebo plus FOLFIRI in patients with previously treated mCRC, but there was a trend toward improved ORR. Pharmacokinetic parameters of trebananib coadministered with FOLFIRI were comparable to those reported for trebananib monotherapy. Although exposures of 5-FU and SN-38 (but not irinotecan) were lower with trebananib coadministration, high data variability limits conclusions about drug–drug interactions. Toxicity of the treatment combination was manageable and AEs, including the distinct toxicity profile of trebananib, were consistent with what has been previously reported for FOLFIRI and trebananib. Although trebananib plus FOLFIRI did not improve PFS in this study, evidence continues to support the concept of antiangiogenesis as a treatment approach in second-line FOLFIRI, including for patients who have previously received angiogenesis inhibitors (Van Cutsem *et al*, 2011). It is possible that treatment approaches incorporating inhibitors of the angiopoietin/Tie2 axis could have a role if administered at different doses/schedules, in less advanced disease and/or if administered in combination with other targeted agents (e.g., VEGF inhibitors). Trebananib plus bevacizumab as first-line therapy in patients with mCRC is currently being evaluated in a phase 2 study (ClinicalTrials.gov, NCT01249521).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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