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 ≥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.
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 angiopeititin-Tie2 receptor axis have distinct roles in the regulation of pathologic angiogenesis (Huang et al, 2010). Evidence suggests that the angiopeititins 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 angiopeititin 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 angiopeititin 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 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 (≥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 ≤1; life expectancy ≥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 2 IV plus 5-FU 400 mg m⁻² 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 ≥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±1 weeks thereafter. Responses were assessed according to RECIST version 1.0 (Therasse et al, 2000) by investigators and confirmed ≥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, placent 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.
RESULTS

Patients. Between December 2008 and May 2010, 144 patients were randomised (Arm A, n = 92; Arm B, n = 49). All but one patient in Arm A received ≥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 had had disease progression before the study was initiated (including arterial and venous thromboembolic events, hypertension, and perforations); generally similar across both treatment arms (Table 1); 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 $C_{\text{max}}$ (221 µg ml$^{-1}$ (69.8%); n = 64) and $C_{\text{ss}}$ (15.6 µg ml$^{-1}$ (52.6%); n = 74) following coadministration with FOLFIRI at week 5 were similar to those reported in the first-in-human phase 1 monotherapy study (236 µg ml$^{-1}$ and 13.7 µg ml$^{-1}$, respectively) (Herbst et al., 2009). Intensive trebananib pharmacokinetic analysis was performed for seven patients (Figure 3A). Among these patients, mean (CV%) steadystate clearance for trebananib was 1.76 ml h$^{-1}$ kg$^{-1}$ (31.0%). At week 5, median (CV%) plasma irinotecan $C_{\text{ss}}$ 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 $C_{\text{ss}}$ 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 ($C_{\text{ss}}$) 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 $C_{\text{ss}}$ 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

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, 0.86; 95% CI, 0.56–1.37; 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 ≥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 ≥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.

- **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.
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).

| Table 1. Baseline demographics and clinical characteristics | Arm A | Arm B |
|-------------------------------------------------------------|-------|-------|
| Trebananib 10 mg kg⁻¹ QW + FOLFIRI (n = 95) |       |       |
| 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) |
| **KRASt 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 KRASt codons 12 and 13 were assessed using the RUO KR-04 KRASt 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.
Assessed for eligibility ($n=187$)

Randomised ($n=144$)

**Arm A**
- Received trebananib 10 mg/kg QW ($n=95$)
- Did not receive trebananib 10 mg/kg QW ($n=1$)

**Arm B**
- Received placebo ($n=49$)
- Did not receive placebo ($n=0$)

Ongoing ($n=14$)
- Discontinued trebananib 10 mg kg$^{-1}$ QW ($n=80$)
- Disease progression ($n=59$)
- Adverse event ($n=27$)
- Death ($n=3$)
- Consent withdrawn ($n=3$)
- Other ($n=1$)

Included in efficacy analysis ($n=95$)
- Excluded from efficacy analysis ($n=0$)
- Included in safety analysis ($n=94$)
- Excluded from safety analysis ($n=1$)

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

| Objective | Arm A | Arm B |
|-----------|-------|-------|
| **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

| Response | Arm A | Arm B |
|----------|-------|-------|
| 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* | 0 (0) | 1 (2) |
| Not done* | 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.

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

*Imaging was not performed at the scheduled tumour assessment.
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 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 $\geq$ 10% of patients in either treatment arm, n (%) | | |
| Diarrhoea | 44 (47) | 20 (41) |
| Nausea | 41 (44) | 18 (37) |
| Neutropenia | 39 (41) | 28 (57) |
| Anemia | 29 (31) | 16 (33) |
| Decreased appetite | 26 (28) | 8 (16) |
| Alopecia | 24 (26) | 18 (37) |
| Fatigue | 23 (24) | 9 (18) |
| Constipation | 20 (21) | 9 (18) |
| Peripheral oedema | 19 (20) | 2 (4) |
| Vomiting | 16 (17) | 19 (39) |
| Abdominal pain | 13 (14) | 5 (10) |
| Pyrexia | 13 (14) | 4 (8) |
| Leucopenia | 12 (13) | 6 (12) |
| Stomatitis | 12 (13) | 6 (12) |
| Cough | 7 (7) | 7 (14) |
| Anaemia | 6 (6) | 12 (24) |

**Table 4. Patient incidence of adverse events of specific interest**

| 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) |
| Venous thromboembolic events | 5 (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) |
| Venous thromboembolic 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) |
| Haemorrhagic events | 1 (1) | 0 (0) |

**Abbreviation:** QW = once weekly.

*Unless otherwise indicated, all adverse events of interest were grade $\leq$ 2.
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| N | * | * | * | * |
|---|---|---|---|---|
| Placebo | Trebananib 10 mg kg⁻¹ QW | Placebo | Trebananib 10 mg kg⁻¹ QW |

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 or placebo plus FOLFIRI. (B) Cmax at week 5 of irinotecan among patients who received trebananib 10 mg kg⁻¹ QW plus FOLFIRI or placebo plus FOLFIRI. (C) Cmax 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 Cmax at week 1 among patients who received placebo plus FOLFIRI by patient sex. (E) Cmax of 5-FU at week 5 among patients who received trebananib 10 mg kg⁻¹ QW plus FOLFIRI or placebo plus FOLFIRI. Cmax = maximum observed concentration; C10 = concentration at steady state; SD = 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 ≥ 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 ≥ 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 angioptetin/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 QW 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 enrolled. 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\(^{-1}\) 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\(^{-1}\) 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 angiogenesis 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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