These may affect physiology (for example, migration rate in GI tract, epithelial cells in GI tract, blood flow rate, etc.), and produce large inter-individual variability in vorinostat pharmacokinetics. Therefore, there is a possibility that the high serum exposures observed in some of the enrolled patients were due to such multiple factors. Patients with metastatic disease were not examined in this trial and the evidence for the effect of vorinostat in patients with metastatic disease is scant. In small studies in patients with metastatic breast cancer, head and neck cancer, and thyroid carcinoma, stand-alone vorinostat was generally well tolerated but led to neither complete nor partial response in any patient, although the stable disease achieved by some patients warrants further research in combination therapy [26–28].

Although efficacy in the treatment of gastric cancer was not a primary objective for this study, 5 patients in group 1 (300 mg bid) achieved stable disease ≥8 weeks, with 1 patient in particular having duration of TTP of 245 days. In contrast, there were two patients in group 2 (400 mg qd) who achieved stable disease ≥8 weeks, possibly due to the lower tolerability observed with this dosing regimen. We observed these results despite the fact that 300 mg bid resulted in lower mean drug exposure compared with 400 mg qd, indicating that the lower drug exposure associated with the 300 mg bid dose level led to greater tolerability with no deleterious effects on efficacy compared with the higher observed drug exposure at the 400 mg qd dose level. Objective responses were not observed in this study. However, considering the cytostatic effect of vorinostat in preclinical models, these data appear to be encouraging [9, 29]. In a previous phase I study in non-Japanese patients, the administration of vorinostat with 300 mg or 400 mg bid for 3 consecutive days followed by 4 days rest regimen showed PR in 2 patients, and stable disease ≥16 weeks in 3 patients out of 13 patients with malignant pleural mesothelioma [30]. Therefore, from a safety and efficacy perspective, this dosing regimen is promising for Japanese patients with GI cancer. Currently, a phase III study is on-going to evaluate 300 mg bid for 3 consecutive days followed by 4 days rest in non-Japanese and Japanese patients with mesothelioma.

When viewing these data, the limitations of the current study should be considered. Specifically, the results from this study are limited due to the small number of patients studied, and further investigation is needed to assess tolerability in a larger patient population. More research is also needed to further characterize the efficacy of vorinostat with regard to whether or not efficacy is dose-dependent and whether differentiated gastric cancer is more responsive to treatment than undifferentiated gastric cancer.

In conclusion, vorinostat given to patients with GI cancer was well tolerated when given 300 mg bid for 3 consecutive days followed by 4 days of rest when compared with 400 mg qd dosing regimen for 21 consecutive days per cycle in Japanese patients. Additionally, 5 patients receiving 300 mg bid and 2 patients receiving 400 mg qd maintained stable disease for >8 weeks, with the maximum duration being 245 days. The current study supports further investigation of vorinostat alone or in combination with other anti-cancer agents in patients with gastric cancer who may be sensitive to epigenetic treatment with an HDAC inhibitor, such as those who exhibit aberrant DNA methylation of p16. Of particular interest will be the evaluation of overlapping hematologic toxicities for the study of a combination approach with other agents with low rates of toxicities. For further study of vorinostat alone, the rate of efficacy will need to be evaluated in a larger population of patients to ensure adequate treatment of gastric cancer.

Conflict of interest Noguchi and Otsuki are employees of MSD K.K., a subsidiary of Merck & Co., Inc., and may own stock or stock options in the company. Mehta is an employee of Merck Sharp & Dohme, Corp., and may own stock or stock options in the company. The other authors report no conflicts of interest.

References

1. Minucci S, Pelicci PG (2006) Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 6:38–51
2. Marks P, Rifkind RA, Richon VM et al (2001) Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 1:194–202
3. Timmermann S, Lehrmann H, Polesskaya A et al (2001) Histone acetylation and disease. Cell Mol Life Sci 58:728–736
4. Wang C, Fu M, Mani S et al (2001) Histone acetylation and the cell-cycle in cancer. Front Biosci 6:D610–29, D610–29
5. Ueno M, Toyota M, Akino K et al (2004) Aberrant methylation and histone deacetylation associated with silencing of SLC5A8 in gastric cancer. Tumour Biol 25:134–140
6. Murai M, Toyota M, Suzuki H et al (2005) Aberrant methylation and silencing of the BNIP3 gene in colorectal and gastric cancer. Clin Cancer Res 11:1021–1027
7. Kawamura YI, Toyota M, Kawashima R et al (2008) DNA hypermethylation contributes to incomplete synthesis of carbohydrate determinants in gastrointestinal cancer. Gastroenterology 135:142–151
8. Marson CM (2009) Histone deacetylase inhibitors: design, structure-activity relationships and therapeutic implications for cancer. Anticancer Agents Med Chem 9:661–692
9. Butler LM, Agus DB, Scher HI et al (2000) Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. Cancer Res 60:5165–5170
10. Duvic M, Talpur R, Ni X et al (2007) Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood 109:31–39
11. Galanis E, Jaeckle KA, Maurer MJ et al (2009) Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study. J Clin Oncol 27:2052–2058
12. Secrist JP, Zhou X, Richon VM (2003) HDAC inhibitors for the treatment of cancer. Curr Opin Investig Drugs 4:1422–1427

13. Richon VM, Garcia-Vargas J, Hardwick JS (2009) Development of vorinostat: current applications and future perspectives for cancer therapy. Cancer Lett 280:201–210
14. Huang C, Ida H, Ito K et al (2007) Contribution of reactivated RUNX3 to inhibition of gastric cancer cell growth following suberoylanilide hydroxamic acid (vorinostat) treatment. Biochem Pharmacol 73:990–1000
15. Gordon KJ, Blobe GC (2008) Role of transforming growth factor-beta superfamily signaling pathways in human disease. Biochim Biophys Acta 1782:197–228
16. Bierie B, Moses HL (2006) Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 6:506–520
17. Ammanamanchi S, Brattain MG (2004) Restoration of transforming growth factor-beta signaling through receptor RI induction by histone deacetylase activity inhibition in breast cancer cells. J Biol Chem 279:32620–32625
18. Kelly WK, O'Connor OA, Krug LM et al (2005) Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol 23:3923–3931
19. Olsen EA, Kim YH, Kuzel TM et al (2007) Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol 25:3109–3115
20. Fujiwara Y, Yamamoto N, Yamada Y et al (2009) Phase I and pharmacokinetic study of vorinostat (suberoylanilide hydroxamic acid) in Japanese patients with solid tumors. Cancer Sci 100:1728–1734
21. Mann BS, Johnson JR, Cohen MH, Justice R et al (2007) FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. Oncologist 12:1247–1252
22. Rubin E, Agrawal N, Friedman E et al (2006) A study to determine the effects of food and multiple dosing on the pharmacokinetics of vorinostat given orally to patients with advanced cancer. Clin Cancer Res 12:7039 doi:10.1158/1078-0432.CCR-06-1802
23. Gehan EA, Tefft MC (2000) Will there be resistance to the RECIST (response evaluation criteria in solid tumors)? J Natl Cancer Inst 92:179–181
24. Dummer R, Cozzio A, Meier S et al (2006) Standard and experimental therapy in cutaneous T-cell lymphomas. J Cutan Pathol 33(Suppl 1):52–57
25. Nishiyama M, Eguchi H (2009) Pharmacokinetics and pharmacogenomics in gastric cancer chemotherapy. Adv Drug Deliv Rev 61:402–407
26. Luu TH, Morgan RJ, Leong L et al (2008) A phase II trial of vorinostat (suberoylanilide hydroxamic acid) in metastatic breast cancer: a california cancer consortium study. Clin Cancer Res 14:7138–7142
27. Woyach JA, Kloos RT, Ringel MD et al (2009) Lack of therapeutic effect of the histone deacetylase inhibitor vorinostat in patients with metastatic radioiodine-refractory thyroid carcinoma. J Clin Endocrinol Metab 94:164–170
28. Blumenschein GR, Jr, Kies MS, Papadimitrakopoulou VA et al (2008) Phase II trial of the histone deacetylase inhibitor vorinostat (Zolinza, suberoylanilide hydroxamic acid, SAHA) in patients with recurrent and/or metastatic head and neck cancer. Invest New Drugs 26:81–87
29. Lobjois V, Frongia C, Jozan S et al (2009) Cell cycle and apoptotic effects of SAHA are regulated by the cellular microenvironment in HCT116 multicellular tumour spheroids. Eur J Cancer 45:2402–2411
30. Krug LM, Curley T, Schwartz L et al (2006) Potential role of histone deacetylase inhibitors in mesothelioma: clinical experience with suberoylanilide hydroxamic acid. Clin Lung Cancer 7:257–261

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ORIGINAL ARTICLE

Phase 1 study of trebananib (AMG 386), an angiogenesis targeting angiopoietin-1/2 antagonist, in Japanese patients with advanced solid tumors

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Abstract

Purpose To evaluate the safety, tolerability, pharmacokinetics, and antitumor activity of trebananib (AMG 386)—a first-in-class angiopoietin-1/2 antagonist peptide-Fc fusion protein—in Japanese patients, we conducted a phase 1, dose escalation study.

Methods Eligible patients were men or women, aged between 20 and 74 years, who had histologically or cytologically confirmed advanced solid tumors refractory to standard treatment. Trebananib (3, 10, and 30 mg/kg) was administered intravenously over 60 min in weekly cycles.

Results From June 2009 to April 2010, a total of 18 patients (6 for each dose cohort) were enrolled into the study. Trebananib was tolerated at all dose levels. No dose-limiting toxicities were observed. The most common adverse events were peripheral edema, constipation, fatigue, and pyrexia. Exposure to trebananib appeared to increase according to the dose administered. Serum clearance appeared to be similar across the dose range with the mean terminal-phase half-life ranging from 93.9 to 95.9 h. No neutralizing antibodies were detected. Tumor response was assessed in 18 patients. Of these, one patient with colon cancer in the 3-mg/kg cohort and one with bladder cancer in the 30-mg/kg cohort had partial responses as their best responses. These 2 patients were on treatment at the time of data cutoff (January 17, 2012).

Conclusion Trebananib was tolerated and showed acceptable safety profile in Japanese patients with advanced solid tumors. The pharmacokinetic profiles were similar to those in the previous studies in the United States. Trebananib also showed evidence of durable antitumor activity in some patients.

Keywords Trebananib · AMG 386 · Angiopoietin 1/2-neutralizing peptibody · Clinical trial, phase 1 · Pharmacokinetics · Safety

Abbreviations

VEGFR	Vascular endothelial growth factor receptor
Ang1	Angiopoietin-1
Ang2	Angiopoietin-2
PK	Pharmacokinetic
ECOG	Eastern Cooperative Oncology Group
AST	Aspartate aminotransferase
ULN	Upper limit of normal
ALT	Alanine aminotransferase
DLT	Dose-limiting toxicity
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
sVCAM-1	Soluble vascular cell adhesion molecule-1
C_{max}	Maximum observed concentration
$AUC_{0\text{-}168}$	Area under the serum concentration–time curve from time 0 to 168 h post-dose

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Introduction

Angiogenesis is an essential process for tumor growth and metastasis [1, 2]. Unless angiogenesis occurs, tumor growth is limited because it is dependent on the continued supply of oxygen [3]. Thus, targeting angiogenesis represents one strategy for the development of anticancer therapies [4], and preclinical models of human cancer have shown that blocking angiogenesis inhibits proliferation of tumor and induces tumor regression [1, 4]. On the basis of these findings, several antiangiogenic agents have been developed and have already been approved for anticancer treatment. These agents include the inhibitors targeting vascular endothelial growth factor receptor (VEGFR) pathway [5, 6], such as monoclonal antibodies and tyrosine kinase inhibitors [7]. However, much attention has been focused on the clinical toxicity profile of these agents [8]. For example, they may increase the risk for several adverse events such as hypertension, proteinuria, coagulation disorders, and gastro-intestinal toxicity [8, 9]. Under these circumstances, newer agents are needed.

One of these candidates is an agent that blocks the interaction of angiopoietins with Tie2 receptor [10, 11]. Angiopoietin-1 (Ang1) is an angiogenic factor that signals through the endothelial cell-specific Tie2 receptor tyrosine kinase [12]. Angiopoietin-2 (Ang2) is expressed only at sites of vascular remodeling, where it reduces vascular integrity and probably makes the endothelial cells more responsive to the proliferative signals of VEGF [12]. In experimental models of cancer, imbalances between Ang1 and Ang2 resulted in a net gain of Ang2 activity, and the over-expression of Ang2 led to enhanced tumor angiogenesis and growth [13]. In addition, dual inhibition of Ang1 and Ang2 resulted in better antitumor activity than inhibition of Ang2 alone, which suggests that dual Ang1/2 inhibition is superior to selective Ang2 inhibition for suppression of angiogenesis in some postnatal settings [14]. Thus, dual Ang1/2 inhibitors are expected to be effective in the treatment of various types of cancer.

Trebananib (AMG 386) is an investigational first-in-class angiopoietin antagonist peptide-Fc fusion protein. It reduces tumor angiogenesis by selectively inhibiting the interaction of Ang1 and Ang2 with the Tie2 receptor [15]. Recently, data from 2 phase 1 studies conducted in the United States became available. In these studies, weekly administration of trebananib showed acceptable safety profile and antitumor activity as monotherapy or in combination with 3 common chemotherapy regimens in patients with advanced solid tumors [16, 17].

However, these studies mainly included Caucasians and it is uncertain whether these findings are generalizable to other ethnic populations such as Asians. Accordingly, we conducted a phase 1 study in Japan. The primary objectives of the study were to evaluate the safety, tolerability, and pharmacokinetic (PK) profile of trebananib in Japanese patients with advanced solid tumors. The secondary objectives were to explore its efficacy and potential biomarkers.

Methods

Study design and ethical considerations

This phase 1, open-label, dose escalation study was conducted at National Cancer Center Hospital East in Japan. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Its protocol was reviewed and approved by the institutional review board of the hospital. All patients provided written informed consent prior to their inclusion in the study.

Patient population

Eligible patients were men or women, aged between 20 and 74 years, who had histologically or cytologically confirmed advanced solid tumor which was refractory to standard treatment or for which no curative treatment was available. Other inclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, normal sinus rhythm on electrocardiographic evaluation, and life expectancy of at least 3 months. Patients were also required to have adequate hematologic, renal, hepatic, and hemostatic function defined as follows: absolute neutrophil count $\geq$1,500/μL; platelet count $\geq$100,000/μL; hemoglobin $\geq$9 g/dL; creatinine clearance >40 mL/min; urinary protein $\leq$30 mg/dL in urinalysis or $\leq$1+ on dipstick; aspartate aminotransferase (AST) $\leq$2.5 times the upper limit of normal (ULN) ($\leq$5 times ULN for patients with liver metastases); alanine aminotransferase (ALT) $\leq$2.5 times ULN ($\leq$5 times ULN for those with liver metastases); alkaline phosphatase $\leq$2.0 times ULN ($\leq$5 times ULN for those with bone or liver metastases); total bilirubin $\leq$2.0 times ULN; and prothrombin time or activated partial thromboplastin time $\leq$1.5 times ULN.

Patients were excluded if they had any central nervous system tumors; hematologic malignancies; unresolved toxicities from prior anticancer therapy; clinically significant cardiovascular disease within 1 year before enrollment such as myocardial infarction, unstable angina, congestive heart failure (New York Heart Association class 2–4), peripheral vascular disease, cerebrovascular disorder, transient ischemic attack, or uncontrolled arrhythmia; uncontrolled hypertension (systolic >150 mm Hg or diastolic >90 mm Hg); a history of arterial or venous thrombosis within 1 year; presence of ascites or pleural

effusion requiring medical intervention; a history of bleeding diathesis or clinically significant bleeding within 6 months; non-healed wound, ulcer, or fracture; head and neck cancer; squamous cell tumor, or lung cancer with large central tumor lesions ≥3 cm; infection with human immunodeficiency virus, hepatitis C virus, or hepatitis B virus; major surgery within 4 weeks; or minor surgical procedure, placement of central venous catheter, or fine needle aspiration within 7 days. Pregnant or breastfeeding women, women of childbearing potential or men having a partner of childbearing potential who were unwilling to use adequate contraceptive precautions during the study were also excluded.

Study treatment

Trebananib was administered intravenously over 60 (±15) min on days 1, 8, 15, and 22 without premedication. Patients were enrolled sequentially into one of 3 dose cohorts (3, 10, and 30 mg/kg; 6 patients for each cohort). The starting dose was 3 mg/kg, which was determined on the basis of the first-in-human study conducted in the United States [16]. Initially, 6 patients received trebananib intravenously every week for up to 28 days, and dose escalation proceeded unless 2 or more patients had a dose-limiting toxicity (DLT) during the first 28 days. Trebananib was not administered on day 29. For patients who had no DLTs and wished to continue the study treatment, trebananib was administered in weekly cycles after day 36.

DLT was defined as any treatment-related toxicity which met the following criteria during the first 28 days according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0: grade 4 or greater hematologic toxicity; grade 3 or greater nonhematologic toxicity other than AST, ALT, and infusion reactions; and AST or ALT >10 times ULN.

If 2 of the initial 6 patients experienced a DLT, additional 3 patients were to be enrolled at that dose level. If at least 3 of 6 patients experienced a DLT, the sponsor (Takeda Bio Development Center Ltd., Tokyo, Japan) was to discuss with the principal investigator—and with the Efficacy and Safety Evaluation Committee, if necessary—to determine whether the dose was intolerable or not.

If patients experienced any DLT during the first 28 days, treatment with trebananib was withheld and the patients were followed up until the resolution of the toxicity. If patients experienced infusion reactions, the infusion was interrupted or the infusion rate was slowed. If the infusion reaction persisted, sequential treatment with antihistamines and steroids was also allowed. Throughout the study, concomitant use of low-dose warfarin (≤1 mg/day) or low molecular weight heparin for prophylaxis of thrombosis was allowed. Other treatments were not allowed during the study except for the supportive care the investigators considered necessary.

Assessments

Medical history was collected within 14 days before enrollment. Patients were hospitalized at least 5 days from day 1. Adverse events were monitored throughout the study and were graded according to the NCI CTCAE version 3.0. Blood pressure, pulse rate, and body temperature were measured at the following time points: predose and 1, 2, 6, 24, 48, and 96 h after starting the initial infusion at week 1; predose and 1 h after starting infusion at weeks 2–4; every week after week 6; and the end-of-study visit (i.e., 4 weeks after the end of treatment). Blood and urine samples for the laboratory tests were collected at the following time points: predose and 24, 48, and 96 h after starting the initial infusion; predose at weeks 2–4; every 4 weeks thereafter; and the end-of-study visit.

Serum samples for PK analysis were collected at the following time points: predose at weeks 1–4; 1, 2, 6, 24, 48, and 96 h after starting infusion at week 1; 1, 2, 6, 24, 48, 96, 168, and 264 h after starting infusion at week 4; every 4 weeks after week 8; and the end-of-study visit. Serum concentration of trebananib was determined by using a validated enzyme-linked immunosorbent assay [16, 17]. PK parameters were estimated by using non-compartmental methods with Phoenix WinNonlin software Version 6.1 (Pharsight Corporation, Mountain View, CA).

Serum samples for the assessment of anti-trebananib antibodies were also collected at the following time points: predose at weeks 1, 2, and 4; every 4 weeks thereafter; the end-of-study visit; and 8 weeks after the end of treatment. In the first analysis of this assessment, the presence/absence of anti-trebananib binding antibodies in serum was confirmed by using a validated acid-dissociation, bridging electrochemiluminescent immunoassay [17, 18]. Thereafter, all serum samples positive for anti-trebananib binding antibodies were evaluated for potential neutralizing capabilities in a validated in vitro receptor binding assay [17].

Furthermore, serum samples were collected for the exploration of a biomarker at predose and 48 h after starting infusion at week 1, predose at weeks 2 and 4, every 4 weeks thereafter, and the end-of-study visit. As a potential biomarker, soluble vascular cell adhesion molecule-1 (sVCAM-1) was quantified by using a specific enzyme-linked immunosorbent assay kit (Quantikine®; R&D Systems Inc., Minneapolis, MN) following the manufacturer's instructions. VCAM-1 is involved in vascular remodeling, and variations in this biomarker may be indicative of a biological response to changes in the vascular endothelium [17].

Table 1 Demographic and baseline characteristics of the study patients

	Trebananib dose cohort			
	3 mg/kg ($n = 6$)	10 mg/kg ($n = 6$)	30 mg/kg ($n = 6$)	Total ($n = 18$)
Sex, *n* (%)				
Male	4 (66.7)	3 (50.0)	3 (50.0)	10 (55.6)
Female	2 (33.3)	3 (50.0)	3 (50.0)	8 (44.4)
Age, years				
Median (range)	57.5 (40–70)	52.5 (47–69)	63.0 (49–66)	57.5 (40–70)
Weight, kg				
Median (range)	55.90 (38.1–64.7)	65.60 (49.6–78.7)	49.65 (47.0–56.0)	55.15 (38.1–78.7)
Primary tumor type, *n* (%)				
Gastric	3 (50.0)	0 (0.0)	3 (50.0)	6 (33.3)
Rectal	1 (16.7)	2 (33.3)	1 (16.7)	4 (22.2)
Pancreatic	1 (16.7)	1 (16.7)	1 (16.7)	3 (16.7)
Colon	1 (16.7)	1 (16.7)	0 (0.0)	2 (11.1)
Bladder	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)
Breast	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Uterine	0 (0.0)	1 (16.7)	0 (0.0)	1 (5.6)
Eastern Cooperative Oncology Group performance status, *n* (%)				
0	6 (100.0)	6 (100.0)	5 (83.3)	17 (94.4)
1	0 (0.0)	0 (0.0)	1 (16.7)	1 (5.6)

Tumor response was evaluated at week 8 and every 8 weeks thereafter by the investigators using computed tomography or magnetic resonance imaging and was classified according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 [19].

Statistical considerations

All data were summarized descriptively. Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as mean combined with standard deviation or median combined with range. All data were analyzed by using SAS® System Version 9.1.3 (SAS Institute, Cary, NC).

Results

From June 2009 to April 2010, a total of 18 patients (6 for each dose cohort) were enrolled into the study. All patients received trebananib and were included in the safety and efficacy analysis. Of these, one patient in the 10-mg/kg cohort discontinued the study treatment because of disease progression during the DLT evaluation period. This patient was excluded from the DLT evaluation. At the time of data cutoff (January 17, 2012), 16 patients ended the study treatment because of disease progression and 2 patients were still receiving treatment. The median number of infusions was 5.5 (range, 4–113) for 3 mg/kg, 6.0 (range, 1–17) for 10 mg/kg, and 6.0 (range, 4–92) for 30 mg/kg. The median cumulative dose was 16.50 mg/kg (range, 12.0–336.7 mg/kg), 60.00 mg/kg (range, 10.0–170.0 mg/kg), and 180.0 mg/kg (range, 120.0–2,760.0 mg/kg), respectively.

Table 1 shows the demographic and baseline characteristics of the study patients. The median age was 57.5 (range, 40–70) years in the total population. Almost all patients (94.4 %) had ECOG performance status of 0. The most common tumor types were gastric ($n = 6$; including 2 patients with gastrointestinal stromal tumors), rectal ($n = 4$; including one with rectal carcinoid), and pancreatic ($n = 3$).

Trebananib was tolerated at all dose levels. All patients had at least one adverse event, but no one discontinued the treatment because of adverse events. No DLTs were observed in any of the dose cohorts. Table 2 shows the common adverse events. The most common adverse events were peripheral edema, constipation, fatigue, and pyrexia. Grade 3 or greater adverse events were reported in 4 patients (one in the 3-mg/kg cohort, one in the 10-mg/kg cohort, and 2 in the 30-mg/kg cohort). Of these, the most frequently reported event was γ-glutamyltransferase increased ($n = 4$). No events with grade 3 or greater were considered treatment-related by the investigator.

Serious adverse events were reported in the following 3 patients: one in the 3-mg/kg cohort (ascites and pleural

Table 2 Common adverse events occurring in at least 3 patients

Preferred term	Trebananib dose cohort							
	3 mg/kg ($n = 6$)		10 mg/kg ($n = 6$)		30 mg/kg ($n = 6$)		Total ($n = 18$)	
	Any	≥Grade 3	Any	≥Grade 3	Any	≥Grade 3	Any	≥Grade 3
Edema peripheral	2 (33)	0 (0)	2 (33)	0 (0)	3 (50)	0 (0)	7 (39)	0 (0)
Constipation	2 (33)	0 (0)	1 (17)	0 (0)	2 (33)	0 (0)	5 (28)	0 (0)
Fatigue	3 (50)	0 (0)	1 (17)	0 (0)	1 (17)	0 (0)	5 (28)	0 (0)
Pyrexia	2 (33)	0 (0)	1 (17)	0 (0)	2 (33)	0 (0)	5 (28)	0 (0)
Anorexia	2 (33)	0 (0)	0 (0)	0 (0)	2 (33)	0 (0)	4 (22)	0 (0)
Diarrhea	1 (17)	0 (0)	1 (17)	0 (0)	2 (33)	0 (0)	4 (22)	0 (0)
ECOG PS worsened	3 (50)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	4 (22)	0 (0)
γ-Glutamyl transferase increased	1 (17)	1 (17)	1 (17)	1 (17)	2 (33)	2 (33)	4 (22)	4 (22)
Hypertension	0 (0)	0 (0)	2 (33)	0 (0)	2 (33)	0 (0)	4 (22)	0 (0)
Abdominal distension	2 (33)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	3 (17)	0 (0)
Ascites	2 (33)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	3 (17)	0 (0)
Cancer pain	1 (17)	0 (0)	1 (17)	0 (0)	1 (17)	0 (0)	3 (17)	0 (0)
Nausea	1 (17)	0 (0)	0 (0)	0 (0)	2 (33)	0 (0)	3 (17)	0 (0)
Rash	1 (17)	0 (0)	1 (17)	0 (0)	1 (17)	0 (0)	3 (17)	0 (0)
Stomatitis	1 (17)	0 (0)	0 (0)	0 (0)	2 (33)	0 (0)	3 (17)	0 (0)

ECOG PS Eastern Cooperative Oncology Group performance status

effusion), one in the 3-mg/kg cohort (subclavian vein thrombosis and cholecystitis), and one in the 30-mg/kg cohort (anorexia). Of these, cholecystitis was considered treatment-related because the patient did not have any complications, such as gallstones, which are known to be a cause of cholecystitis. Other events were not considered treatment-related by the investigator. Subclavian vein thrombosis was considered to be related to the central venous catheter that was placed in the patient.

Figure 1 shows serum concentration–time profiles of trebananib. The serum concentration of trebananib gradually declined after the completion of 1-hour infusion. After 4 once-weekly infusions, the serum concentrations increased slightly compared with those after the initial infusion. Table 3 shows the PK parameters of trebananib. Exposure to trebananib (maximum observed concentration [C_{max}] and area under the serum concentration–time curve from time 0 to 168 h post-dose [$AUC_{0\text{-}168}$]) on both weeks 1 and 4 appeared to increase according to the dose administered. Serum clearance appeared to be similar across the dose levels with the mean total clearance ranging from 1.44 to 1.71 mL/h/kg. The mean terminal-phase

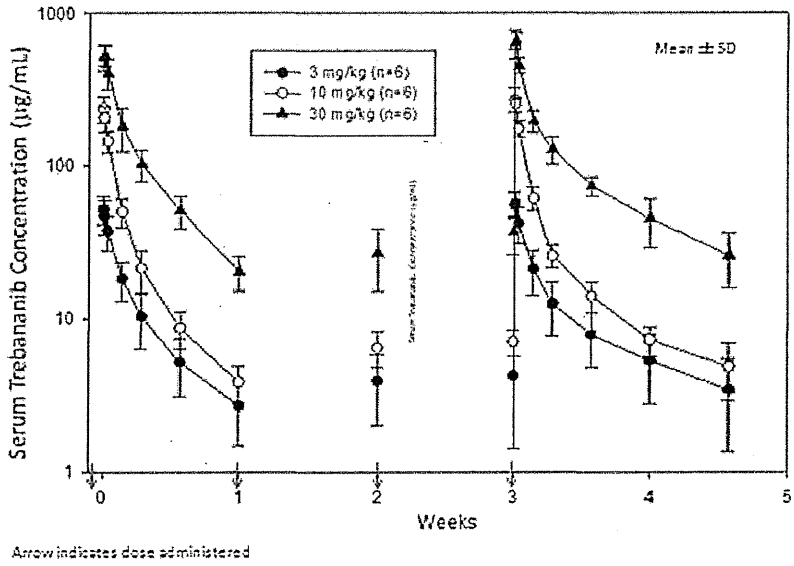

Fig. 1 Serum concentration–time curves of trebananib

Table 3 Pharmacokinetic parameters of trebananib

Week 1			Week 4							
T_{max} (h)	C_{max} (μg/mL)	$AUC_{0\text{-}168}$ (h*μg/mL)	T_{max} (h)	C_{max} (μg/mL)	$AUC_{0\text{-}168}$ (h*μg/mL)	$t_{1/2,\ z}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)	$AUC_{0\text{-}168}$ AR	C_{min} (μg/mL)
3 mg/kg										
$n = 6$			$n = 6$							
1.07 (1.03–1.08)	52.3 (11.3)	1,760 (582)	1.07 (1.02–2.03)	59.0 (10.1)	2,170 (715)	95.9 (35.1)	1.50 (0.423)	158 (49.0)	1.24 (0.0514)	5.32 (2.54)
10 mg/kg										
$n = 6$			$n = 5$							
1.03 (1.02–1.05)	239 (47.1)	4,630 (925)	1.02 (1.02–2.02)	277 (48.8)	5,880 (560)	95.4 (14.8)	1.71 (0.165)	121 (22.2)	1.19 (0.0686)	7.27 (1.52)
30 mg/kg										
$n = 6$			$n = 6$							
1.17 (1.02–2.00)	551 (86.8)	18,000 (4,490)	1.51 (1.03–2.02)	689 (105)	21,200 (2,910)	93.9 (25.6)	1.44 (0.191)	137 (30.3)	1.21 (0.214)	45.1 (16.2)

All parameters are reported as mean (standard deviation) values, except for T_{max}, which is reported as a median (range) value

$AUC_{0\text{-}168}$ = The area under serum concentration–time curve from time 0 to 168 h post-dose, $AUC_{0\text{-}168}$ AR = The $AUC_{0\text{-}168}$ accumulation ratio (=[$AUC_{0\text{-}168}$ on week 4]/[$AUC_{0\text{-}168}$ on week 1]), CL = The apparent total clearance (=[actual dose]/[$AUC_{0\text{-}168}$ on week 4]), C_{max} = The maximum observed serum concentration after dosing, C_{min} = The serum concentration at 168 h after dosing, $t_{1/2,\ z}$ = The estimated terminal-phase half-life (=ln(2)/λz, where λz is the terminal rate constant estimated via linear regression of the terminal log-linear decay phase), T_{max} = The time at which C_{max} was observed, V_{ss} = The volume of distribution at steady state (=MRT*CL, where MRT is the mean residence time.)

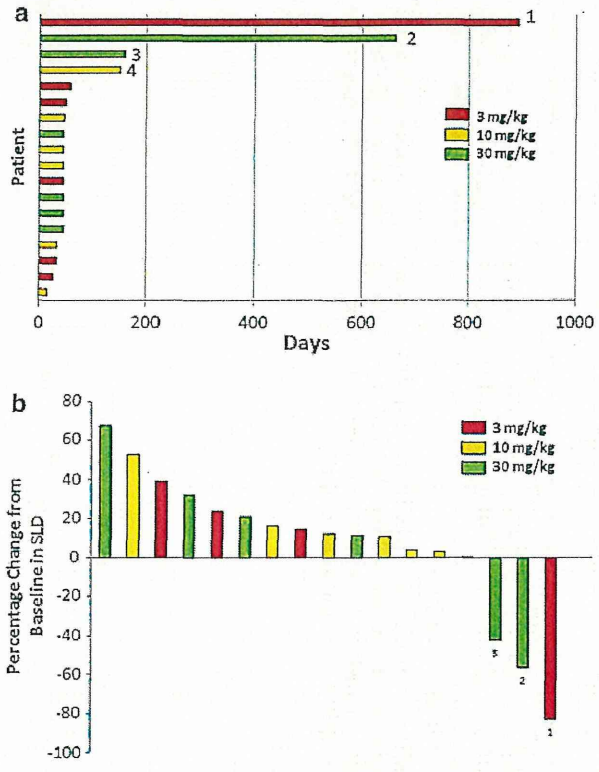

Fig. 2 Antitumor activity of trebananib. **a** Time to disease progression. Tumor type: *1* Colon, *2* Bladder, *3* Stomach (gastrointestinal stromal tumor), *4* Pancreas. **b** The maximum percent change in target lesions. *SLD* sum of the longest diameter. Tumor type: *1* Colon, *2* Bladder, *3* Stomach (gastrointestinal stromal tumor). One patient with colon cancer in the 3-mg/kg cohort and one with bladder cancer in the 30-mg/kg cohort had a best response of partial response

half-life ranged from 93.9 to 95.9 h. Minimal accumulation was observed after multiple dosing with approximate 1.2 of the accumulation ratio of $AUC_{0\text{-}168}$.

Anti-trebananib binding antibodies were detected in 2 patients at 3 mg/kg and one at 10 mg/kg. However, no neutralizing antibodies were observed in their serum samples. Concentrations of sVCAM-1 transiently increased after the infusion according to the dose administered (data not shown).

Figure 2 shows the antitumor activity of trebananib. All patients had measurable diseases at baseline. One patient with colon cancer in the 3-mg/kg cohort and one with bladder cancer in the 30-mg/kg cohort had a best response of partial response. These 2 patients were on treatment at the time of data cutoff. The longest treatment period was over 2 years in the patient with colon cancer (Fig. 2a). One of 18 patients underwent computed tomography examination without receiving contrast agent at post-dose. Therefore, the tumor regions were not comparable between baseline and post-dose. As a result, 17 patients were included in the maximum percentage change in target lesions (Fig. 2b). No clinically meaningful relationship was observed between the concentrations of sVCAM-1 and tumor responses (data not shown).

Discussion

Results of our study show that weekly infusions of trebananib up to 30 mg/kg were tolerated without any treatment discontinuation because of adverse events. Adverse events were mild to moderate in most patients. No DLTs were observed. These results are consistent with those of the phase 1 single-agent study conducted in the United States [16]. In our study, the most common toxicities included peripheral edema and fatigue, which were also observed in the study conducted in the United States [16]. Of these, peripheral edema is a unique adverse event that has been considered to be related to trebananib [20]. No unexpected toxicities were reported.

The safety profile of trebananib was different from that of the VEGF/VEGFR pathway inhibitors, although both agents inhibit angiogenesis. Of the common toxicities associated with the VEGF-axis inhibitors, hypertension is the most prominent adverse event because the VEGF/VEGFR pathway is a regulator of vasodilatation [8, 9]. For example, grade 3/4 hypertension occurred in 4–21 % of patients who received the VEGF-axis inhibitors in the previous studies [21–23]. It is also a frequent reason to delay treatment [9]. In our study, although 4 patients experienced hypertension, these events were mild to moderate and did not require treatment discontinuation. No grade 3/4 hypertension was reported. Other common toxicities associated with VEGF-axis inhibitors such as proteinuria, hemorrhage, or thrombosis did not occur. Although subclavian vein thrombosis was reported in one patient, this event was considered to be related to the central venous catheterization. These distinct safety profiles of trebananib and the VEGF-axis inhibitors are probably derived from the fact that both agents inhibit angiogenesis in a completely different pathway and suggest that they may be combined to improve efficacy without significant overlapping toxicities.

In the PK data of our study, dose-dependent exposure and minimal accumulation of trebananib after 4 once-weekly infusions were observed. These results are consistent with those of the phase 1 studies in the United States [16, 17], and estimated values of PK parameters were similar among the studies. For example, the mean serum clearance ranged from 1.44 to 1.71 mL/h/kg in our study, whereas it ranged from 0.70 to 1.27 mL/h/kg in the previous single-agent study [16]. In addition, the mean C_{max}

after 4 once-weekly infusions of 10-mg/kg trebananib was 277 μg/mL in our study, 249 μg/mL in the single-agent study [16], and 219 μg/mL in the study combined with chemotherapies [17]. These results suggest the absence of ethnic difference in the PK profile of trebananib when intravenously administered weekly.

Although anti-trebananib binding antibodies were detected in 3 patients in our study, no neutralizing antibodies were detected. The previous studies have provided similar results and have also shown that the anti-trebananib antibodies had no apparent effect on serum trebananib concentrations [16, 17]. From these results, we consider that the immune response induced by multiple dosing of trebananib is unlikely to affect the exposure.

In the efficacy analysis, trebananib showed evidence of antitumor activity. Two patients, one with colon cancer and the other with bladder cancer, achieved a partial response. Both of them had a durable partial response and were on treatment at the time of data cutoff. In the previous single-agent study conducted in the United States, of 29 patients with evaluable tumor response, one patient with advanced ovarian cancer refractory to multiple chemotherapies had a partial response with the dose of 30 mg/kg [16]. These results suggest the efficacy of trebananib as monotherapy. Although concentrations of sVCAM-1 transiently increased in a dose-dependent manner, no clinically meaningful relationship was observed between the concentrations of sVCAM-1 and tumor responses. Further efforts may be warranted, because selecting suitable biomarkers for angiopoietin/Tie2 axis is still challenging [17].

In conclusion, trebananib was tolerated and showed acceptable safety profile in Japanese patients with advanced solid tumors. These results are consistent with those of the phase 1 single-agent study conducted in the United States. The PK parameters in Japanese were also similar to those obtained in the previous studies in the United States. These results suggest the absence of ethnic difference. Furthermore, trebananib showed evidence of durable antitumor activity in some patients. To confirm the favorable profiles of trebananib, further clinical trials including randomized controlled trials are needed. At present, several trials that evaluate the efficacy and safety of trebananib in combination with either VEGF-axis inhibitors or chemotherapies are in progress [24]. These programs include 3 phase 3 clinical trials in patients with ovarian cancer (TRINOVA-1, TRINOVA-2 and TRINOVA-3; ClinicalTrials.gov NCT01204749, NCT01281254 and NCT 01493505, respectively).

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Conflict of interest This study was sponsored by Takeda Bio Development Center Ltd. Kazuhiro Shibayama and Takatoshi Takubo are employees of Takeda Bio Development Center Ltd. David M. Weinreich was an employee as leadership position and owned stock of Amgen Inc. The other authors have no conflict of interest.

References

1. Fidler IJ, Kerbel RS, Ellis LM (2001) Biology of cancer: angiogenesis. In: Devita VT, Hellman S, Rosenberg SA (eds) Cancer: principles and practice of oncology. Lippincott-Raven Publishers, Philadelphia, pp 137–147
2. Leek RD (2001) The prognostic role of angiogenesis in breast cancer. Anticancer Res 21:4325–4331
3. Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med 6:389–395
4. Scappaticci FA (2002) Mechanisms and future directions for angiogenesis-based cancer therapies. J Clin Oncol 20:3906–3927
5. Dvorak HF (2002) Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol 20:4368–4380
6. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9:669–676
7. Veeravagu A, Hsu AR, Cai W, Hou LC, Tse VC, Chen X (2007) Vascular endothelial growth factor and vascular endothelial growth factor receptor inhibitors as anti-angiogenic agents in cancer therapy. Recent Pat Anticancer Drug Discov 2:59–71
8. Eskens FA, Verweij J (2006) The clinical toxicity profile of vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR) targeting angiogenesis inhibitors: a review. Eur J Cancer 42:3127–3139
9. Roodhart JM, Langenberg MH, Witteveen E, Voest EE (2008) The molecular basis of class side effects due to treatment with inhibitors of the VEGF/VEGFR pathway. Curr Clin Pharmacol 3:132–143
10. Yu Y, Varughese J, Brown LF, Mulliken JB, Bischoff J (2001) Increased Tie2 expression, enhanced response to angiopoietin-1, and dysregulated angiopoietin-2 expression in hemangioma-derived endothelial cells. Am J Pathol 159:2271–2280
11. Bach F, Uddin FJ, Burke D (2007) Angiopoietins in malignancy. Eur J Surg Oncol 33:7–15
12. Maisonpierre PC, Suri C, Jones PF et al (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277:55–60
13. Ahmad SA, Liu W, Jung YD et al (2001) The effects of angiopoietin-1 and -2 on tumor growth and angiogenesis in human colon cancer. Cancer Res 61:1255–1259
14. Coxon A, Bready J, Min H et al (2010) Context-dependent role of angiopoietin-1 inhibition in the suppression of angiogenesis and tumor growth: implications for AMG 386, an angiopoietin-1/2-neutralizing peptibody. Mol Cancer Ther 9:2641–2651
15. Oliner J, Min H, Leal J et al (2004) Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. Cancer Cell 6:507–516
16. Herbst RS, Hong D, Chap L et al (2009) Safety, pharmacokinetics, and antitumor activity of AMG 386, a selective angiopoietin inhibitor, in adult patients with advanced solid tumors. J Clin Oncol 27:3557–3565

17. Mita AC, Takimoto CH, Mita M et al (2010) Phase 1 study of AMG 386, a selective angiopoietin 1/2-neutralizing peptibody, in combination with chemotherapy in adults with advanced solid tumors. Clin Cancer Res 16:3044–3056
18. Zhong ZD, Dinnogen S, Hokom M et al (2010) Identification and inhibition of drug target interference in immunogenicity assays. J Immunol Methods 355:21–28
19. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumours. J Natl Cancer Inst 92:205–216
20. Karlan BY, Oza AM, Richardson GE et al (2012) Randomized, double-blind, placebo-controlled phase II study of AMG 386 combined with weekly paclitaxel in patients with recurrent ovarian cancer. J Clin Oncol 30:362–371
21. Hurwitz H, Fehrenbacher L, Novotny W et al (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335–2342
22. Escudier B, Eisen T, Stadler WM et al (2007) Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 356: 125–134
23. Hecht JR, Trarbach T, Hainsworth JD et al (2011) Randomized, placebo-controlled, phase III study of first-line oxaliplatin-based chemotherapy plus PTK787/ZK 222584, an oral vascular endothelial growth factor receptor inhibitor, in patients with metastatic colorectal adenocarcinoma. J Clin Oncol 29:1997–2003
24. Robson EJ, Ghatage P (2011) AMG 386: profile of a novel angiopoietin antagonist in patients with ovarian cancer. Expert Opin Investig Drugs 20:297–304

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PHASE I STUDIES

A phase I study of intravenous aflibercept with FOLFIRI in Japanese patients with previously treated metastatic colorectal cancer

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Summary Aflibercept, a recombinant fusion protein, is a potent inhibitor of vascular endothelial growth factor (VEGF)-A, VEGF-B, and the placental growth factor (PlGF). The present study was an open-label, sequential-cohort, dose-escalation trial of intravenous aflibercept administered every 2 weeks in combination with 5-fluorouracil, levofolinate, and irinotecan (FOLFIRI) in patients with previously treated metastatic colorectal cancer (mCRC). We aimed to assess the safety, dose-limiting toxicities (DLTs), pharmacokinetics, and preliminary efficacy of the combination therapy to determine the recommended phase II dose (RPTD) for Japanese patients. Two doses of aflibercept (2.0 and 4.0 mg/kg) were set, and DLTs were evaluated in the first 2 cycles. The subjects comprised 16 patients (n=3 and 13 for 2.0 and 4.0 mg/kg aflibercept, respectively) who received a total of 149 cycles of aflibercept with FOLFIRI. No DLTs were observed at both doses. The frequent adverse events encountered were leukopenia, neutropenia, anemia, diarrhea, fatigue, decreased appetite, stomatitis, dysphonia, nausea, and epistaxis. The most common grade 3/4 adverse events were neutropenia for both doses and hypertension for the 4.0 mg/kg dose. Free aflibercept exposure increased with the dose, whereas exposure to VEGF-bound aflibercept remained similar at both doses. The response rate and progression-free survival at 4.0 mg/kg was 8.3 % and 7.59 months, respectively. In conclusion, the combination of aflibercept and FOLFIRI was well tolerated at both doses. The RPTD of aflibercept in combination with FOLFIRI for Japanese patients with mCRC was determined to be 4.0 mg/kg every 2 weeks.
ClinicalTrials.gov identifier: NCT00921661

Keywords Aflibercept (VEGF trap) · Vascular endothelial growth factor (VEGF) · FOLFIRI · Metastatic colorectal cancer · Phase I study

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Introduction

Angiogenesis is a physiological process generating new blood vessels from preexisting vessels. Aberrant angiogenesis is associated with many pathological conditions, including tumor growth and metastasis [1–5]. Vascular endothelial growth factor (VEGF), a powerful mitogen for endothelial cells, promotes the formation of new blood vessels and is required for the growth of both normal and tumor tissues [1,

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6]. VEGF is one of the most potent angiogenic factors involved in tumor-induced angiogenesis [7, 8]. Inhibition of angiogenesis by agents that block VEGF has been validated as an effective antitumor therapy for the treatment of multiple tumor types, including metastatic colorectal cancer (mCRC) [9]. Aflibercept (also known as VEGF Trap) is a recombinant fusion protein in which the extracellular domains of human VEGF receptors (VEGFRs) 1 and 2 are fused to the Fc portion of human immunoglobulin G1 [10]. Aflibercept binds with all isoforms of VEGF-A, with an affinity higher than that of the endogenous receptor primarily involved in angiogenesis (VEGFR-2). In addition, aflibercept binds to other VEGF family members, VEGF-B and the placental growth factor (PlGF) [11]. PlGF has been implicated in pathological angiogenesis, and its blocking shows an antitumor effect in animal models [12, 13]. Preclinical studies have shown that aflibercept can effectively suppress tumor vascularization and the growth of various tumor types [10]. These results suggest that aflibercept may offer an additional benefit in treating malignant disease.

Colorectal cancer (CRC) is the second most frequent cancer and the second leading cause of cancer death in developed countries [14]. Approximately 50 % of patients with CRC develop metastatic disease [15]. Mortality from CRC accounts for 12 % of all cancer deaths in Japan [16]. In the United States, Europe, and Japan, the standard chemotherapies for mCRC in the first- and second-line settings are FOLFOX (fluorouracil/leucovorin/oxaliplatin), CapeOX (capecitabine/oxaliplatin), and FOLFIRI. The combinations of molecular-targeted drugs, such as bevacizumab, cetuximab, and panitumumab, with the above mentioned standard chemotherapy regimens have been demonstrated to offer additional benefits; therefore, this approach is now incorporated into treatment guidelines. A recent multinational phase III study showed that aflibercept in combination with FOLFIRI as second-line treatment improved the survival of patients with mCRC [17]. Aflibercept is the first molecular-targeted drug to show a survival benefit in combination with FOLFIRI in the second-line setting.

The present phase I study (ClinicalTrials.gov identifier: NCT00921661) was conducted to assess the safety, dose-limiting toxicities (DLTs), pharmacokinetics, and preliminary efficacy of intravenous aflibercept in combination with FOLFIRI in previously treated patients with mCRC to determine its RPTD for Japanese patients.

Materials and methods

Patient eligibility

Eligible patients were men or non-pregnant women aged ≥20 years, who had histologically or cytologically proven metastatic unresectable colorectal adenocarcinomas and had undergone at least one prior chemotherapy. All the eligible patients provided written consent for participation in the study before the initiation of any study-specific procedures. Patients were excluded from the study if they met any of the following criteria: anticancer therapy within 28 days before the study; unresolved toxicity from prior anticancer therapy; Eastern Cooperative Oncology Group (ECOG) performance status >1; uncontrolled malignant ascites; central nervous system involvement; severe heart disease (eg, myocardial infarction, unstable angina, and New York Heart Association [NYHA] class III or IV congestive heart failure); active human immunodeficiency virus (HIV) or hepatitis B or C infection; severe acute or chronic medical condition(s); inadequate bone marrow function (neutrophil count$<1.5\times10^9$/L, platelet count$<100\times10^9$/L, or hemoglobin<9.0 g/dL); inadequate liver function (total bilirubin> 1.5×the upper limit of the normal range [ULN] and aspartate aminotransferase or alanine aminotransferase>2.5× ULN); inadequate renal function (creatinine>1.5×ULN or creatinine clearance<60 mL/min); urine protein-creatinine ratio >1 or urinary protein excretion >500 mg/24 h; uncontrolled hypertension of >150/100 mm Hg; uncontrolled thromboembolic event, active bleeding, or coagulopathy; and hypersensitivity to recombinant proteins or components of FOLFIRI.

Study design

The present study was designed as an open-label, sequential-cohort, dose-escalation, phase I trial conducted at 3 clinical institutes in Japan. It was approved by the institutional review board of all the participating institutes and was conducted in accordance with the ethical principles of the Declaration of Helsinki and the Japanese Good Clinical Practice guidelines.

Drug dose and administration

This study consisted of 2 phases: the dose-escalation and expansion phases. During the dose-escalation phase, aflibercept administration was started at 2.0 mg/kg (dose 1) and then increased to 4.0 mg/kg (dose 2) in a stepwise manner. Three to 6 patients initially received dose 1, and then another 3 to 6 patients received dose 2 if no DLT was observed in the first 3 patients or if only 1 of the 6 patients experienced DLT at dose 1. In the expansion phase, 10 additional patients received the highest dose at which <33 % of the patients developed DLTs in the dose-escalation phase. The RPTD was determined on the basis of the presence of a DLT incidence rate of <33 %, overall safety, and pharmacokinetic analysis. Based on the RPTD of aflibercept for non-Japanese patients indicated by a previous study, we used

4.0 mg/kg once every 2 weeks as the optimum dose. The same dose was also adopted for the global phase III study of the FOLFIRI combination [17].

Aflibercept was administered by intravenous infusion in combination with FOLFIRI every 2 weeks. On day 1 of each cycle, the patients received the following drugs: aflibercept, intravenous infusion at dose 1 or 2 for 1 h; irinotecan, 90-min infusion at 150 mg/m^2 and co-administered with levofolinate infusion (200 mg/m^2) for 2 h; and 5-fluorouracil (5-FU), bolus administration at 400 mg/m^2 for 2–4 min, followed by a 46-h continuous infusion at 2400 mg/m^2. Antiemetic premedication was administered together with serotonin 5-HT$_3$ receptor antagonist and dexamethasone before the initiation of aflibercept infusion.

Definition of DLT

A DLT was defined as any of the following toxicities occurring during the first 2 cycles (cycles 1 and 2): grade 3 or 4 neutropenia complicated by fever (≥38.5 °C) or infection; grade 4 neutropenia persisting for >7 days; grade 4 thrombocytopenia or grade 3 thrombocytopenia complicated by hemorrhage; grade 4 non-hematologic toxicities; grade 3 non-hematologic toxicities other than fatigue, anorexia, nausea, vomiting, or hyponatremia; uncontrolled hypertension or proteinuria; and symptomatic arterial thromboembolic events.

Safety and efficacy assessments

Safety was assessed by monitoring the incidence and severity of the adverse events and abnormal laboratory results. The adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) ver. 3.0. Antitumor response was evaluated at baseline and on day 14 of every 3 cycles according to the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.0 [18]. Response rate was defined as the proportion of patients with complete response (CR) or partial response (PR) in the analysis population. Disease control rate (DCR) was defined as the proportion of patients with CR, PR, or stable disease (SD) for at least 8 weeks from the initiation of the study regimen. Progression-free survival (PFS) was defined as the time from the registration to the date of disease progression (documented tumor progression or clinical progression/symptomatic deterioration) or death from any cause. The Kaplan-Meier method was used to estimate the PFS. The 95 % confidence interval (CI) for the median PFS was calculated using the method described by Brookmeyer and Crowley [19].

Pharmacokinetics and immunogenicity

Free and VEGF-bound aflibercept concentrations were measured using a specific enzyme-linked immunosorbent assay. Blood samples were obtained at the following time points: before and at 1, 3, 7, 23, 47, 167 (day 8), and 335 h (day 15) after the end of aflibercept infusion in cycle 1; before aflibercept infusion on day 1 of each cycle from cycle 2 onward; and 30 and 90 days after the end of the treatment cycles. For plasma irinotecan concentrations, blood samples were obtained before and at 1.5, 2.0, 4.5, and 23 h after the initiation of irinotecan infusion in cycle 1. For plasma 5-FU concentrations, blood samples were obtained before infusion of aflibercept and at 2.5, 21, and 45 h after the bolus administration of 5-FU in cycle 1. The pharmacokinetic parameters were as follows: for free and VEGF-bound aflibercept, the parameters were concentration at trough (C_{trough}), maximum plasma concentration (C_{max}), area under the concentration time curve (AUC), clearance (CL), distribution volume at steady state (V_{ss}), terminal half-life ($t_{1/2}$); for irinotecan and its active metabolite SN-38, the parameters were CL and AUC; and for 5-FU, the parameter was the concentration at steady state during continuous infusion.

To examine the potential immunogenicity of aflibercept, anti-aflibercept antibody levels were measured before infusion on day 1 of each cycle, at the end of the treatment, and at 90 d after the last study treatment.

Results

Patient characteristics

Sixteen patients were enrolled in this study, of whom 3 received dose 1 and 13 received dose 2. Ten patients in dose 2 were included for the expansion phase. The baseline characteristics of all the patients are presented in Table 1. The median patient age was 57 years. All the patients had an ECOG performance status of 0 or 1, and the primary tumor site was the colon or rectum (8 patients each). The patients had previously undergone a median of 1 chemotherapy regimen (range, 1–3), and all the 16 patients had been treated with 5-FU; 15 (93.8 %), with oxaliplatin; 10 (62.5 %), with bevacizumab; and 3 (18.8 %), with irinotecan. The data of all the 16 patients were included in the safety, efficacy, and pharmacokinetic analyses. One patient was excluded from DLT evaluation because the patient did not receive cycle 2 of the aflibercept therapy due to consent withdrawal not associated with adverse event.

Safety and tolerability

The median numbers of cycles administered were 6 (range, 3–9) and 10 (range, 1–23) and the total numbers of cycles were 18 and 131 for doses 1 and 2, respectively. For all the 3 patients receiving dose 1 and 11 of the 13 patients receiving dose 2, treatment was discontinued because of disease

Table 1 Baseline patient characteristics

Characteristics	Dose level		
	2 mg/kg (*N*=3)	4 mg/kg (*N*=13)	ALL (*N*=16)
Median age [range] (years)	59.0 [51–66]	55.0 [47–69]	57.0 [47–69]
Gender [n (%)]			
Male	1 (33.3 %)	9 (69.2 %)	10 (62.5 %)
Female	2 (66.7 %)	4 (30.8 %)	6 (37.5 %)
Median weight [range] (kg)	51.5 [50.6–71.0]	62.9 [36.4-86.2]	61.3 [36.4–86.2]
ECOG PS[a] [n(%)]			
0	2 (66.7 %)	7 (53.8 %)	9 (56.3 %)
1	1 (33.3 %)	6 (46.2 %)	7 (43.8 %)
Primary tumor site [n(%)]			
Colon	2 (66.7 %)	6 (46.2 %)	8 (50.0 %)
Rectum	1 (33.3 %)	7 (53.8 %)	8 (50.0 %)
Median lines of prior chemotherapy [range]	2.0 [2, 3]	1.0 [1, 2]	1.0 [1–3]
Prior chemotherapy [n(%)]			
5-FU	3 (100 %)	13 (100 %)	16 (100 %)
Irinotecan	2 (66.7 %)	1 (7.7 %)	3 (18.8 %)
Oxaliplatin	2 (66.7 %)	13 (100 %)	15 (93.8 %)
Bevacizumab	3 (100 %)	7 (53.8 %)	10 (62.5 %)

[a]Performance status

progression. With regard to the remaining 2 patients receiving dose 2, 1 requested discontinuation of the treatment in order to receive an alternative treatment, whereas the other patient was still receiving the treatment at the time of data cutoff. The aflibercept dose was reduced once in 1 patient receiving dose 2 at the discretion of the investigator owing to grade 2 proteinuria. The treatment cycle was delayed for >2 d in 80 % and 56 % of cycles at doses 1 and 2, respectively. Accordingly, the mean cycle duration was 3.2 and 2.9 weeks, and the mean relative dose intensities for aflibercept were 67 % and 73 % at doses 1 and 2, respectively.

No DLTs were observed at both doses during the first 2 cycles. All the patients experienced at least one adverse event. The incidence rates of the common adverse events are summarized in Table 2. The frequently reported adverse events at dose 1 were leukopenia, neutropenia, anemia, diarrhea, nausea, vomiting, and fatigue; and those at dose 2 were leukopenia, neutropenia, anemia, diarrhea, fatigue, decreased appetite, stomatitis, dysphonia, and nausea. Of these, the most frequent grade 3/4 adverse event was neutropenia at both doses. Serious adverse events were observed after the DLT evaluation period in 2 patients at dose 2. One patient experienced 2 episodes of grade 3 febrile neutropenia during cycles 3 and 10; and grade 3 anemia, grade 4 thrombocytopenia, and grade 3 dehydration during cycle 10. All the adverse events were resolved, except for anemia, which was observed to be improving at the last follow-up examination. The other patient had a grade 4 hepatic function abnormality due to disease progression. There was no adverse event resulting in death or treatment discontinuation. The most frequent adverse event leading to dose reduction or cycle delay was neutropenia, which was observed in 100 % and 92.3 % of the patients at doses 1 and 2, respectively. Toxicities commonly associated with anti-angiogenesis agents, such as dysphonia, epistaxis, hypertension, and proteinuria, were also observed. The most frequent grade 3/4 adverse event associated with anti-angiogenesis agents was hypertension, which was observed only at dose 2.

Pharmacokinetics and immunogenicity

A graph of the mean plasma concentration plotted against time for free and VEGF-bound aflibercept is presented in Fig. 1. At both doses, the plasma concentration of free aflibercept was highest on day 1 after infusion and decreased thereafter during the 2 weeks of cycle 1. The plasma concentration of VEGF-bound aflibercept exhibited a gradual increase. The plasma concentration of free aflibercept was higher at dose 2 than at dose 1, whereas those of VEGF-bound aflibercept were similar at both doses. The pharmacokinetic parameters of free and VEGF-bound aflibercept are summarized in Table 3. The exposure to free aflibercept increased with the dose, with the mean C_{max} being 41.0 and 72.7 μg/mL and mean AUC being 140 and 269 μg·d/mL at doses 1 and 2, respectively. In contrast, the exposure to VEGF-bound aflibercept at dose 1 was similar to that at dose 2, with the mean C_{max} being 1.94 and 1.86 μg/mL and mean AUC being 14.0 and 13.0 μg d/mL at the 2 respective

Table 2 Incidence of common all-causality adverse events[a] by worst grade

Preferred term	Dose level			
	2 mg/kg (*N*=3)		4 mg/kg (*N*=13)	
	All grades n (%)	Grades 3, 4 n (%)	All grades n (%)	Grades 3, 4 n (%)
Hematological toxicities[b]				
Leukopenia	3 (100 %)	1 (33.3 %)	13 (100 %)	8 (61.5 %)
Neutropenia	3 (100 %)	3 (100 %)	12 (92.3 %)	10 (76.9 %)
Anemia	3 (100 %)	0	11 (84.6 %)	1 (7.7 %)
Thrombocytopenia	2 (66.7 %)	0	9 (69.2 %)	1 (7.7 %)
Non-hematological toxicities				
Diarrhoea	3 (100 %)	0	12 (92.3 %)	1 (7.7 %)
Fatigue	3 (100 %)	0	12 (92.3 %)	0
Decreased appetite	2 (66.7 %)	0	12 (92.3 %)	0
Stomatitis	2 (66.7 %)	0	11 (84.6 %)	2 (15.4 %)
Dysphonia	2 (66.7 %)	0	11 (84.6 %)	0
Nausea	3 (100 %)	0	11 (84.6 %)	0
Epistaxis	1 (33.3 %)	0	10 (76.9 %)	0
Hypertension	0	0	8 (61.5 %)	4 (30.8 %)
Alopecia	2 (66.7 %)	0	8 (61.5 %)	0
Vomiting	3 (100 %)	0	7 (53.8 %)	0
Headache	1 (33.3 %)	0	6 (46.2 %)	0
Nasopharyngitis	1 (33.3 %)	0	5 (38.5 %)	0
Abdominal pain	1 (33.3 %)	0	5 (38.5 %)	0
Constipation	0	0	5 (38.5 %)	0
Gingivitis	0	0	5 (38.5 %)	0
Pyrexia	1 (33.3 %)	0	4 (30.8 %)	0

[a]Events listed are those occurring in ≥4 patients at 4 mg/kg at any grade.

[b]Based on laboratory data.

doses. These findings suggest that the binding saturation of endogenously produced VEGF to aflibercept was achieved at both levels. The ratio of free aflibercept concentration to VEGF-bound aflibercept concentration at the trough (2 weeks after aflibercept administration) was >1 throughout the treatment duration at dose 2 (Fig. 2), indicating that dose 2 (4.0 mg/kg) was sufficient for providing a free aflibercept concentration in excess of that of VEGF-bound aflibercept throughout the treatment duration.

None of the 13 patients who were assessed for immunogenicity had positive results for anti-aflibercept antibodies. No major allergic reactions were observed in this study.

Anti-tumor activity

Three and 12 patients receiving doses 1 and 2, respectively, underwent tumor response evaluation according to the RECIST criteria. The response rates were 0 % and 8.3 % at doses 1 and 2, respectively, with 1 case of PR at dose 2. The DCRs were 66.7 % and 75.0 % at doses 1 and 2, respectively. The PFS at dose 2 was 7.59 months (95 % CI, 2.33–9.59).

Discussion

To our knowledge, this is the first trial of the combination of aflibercept and FOLFIRI in Japan. Our results showed that

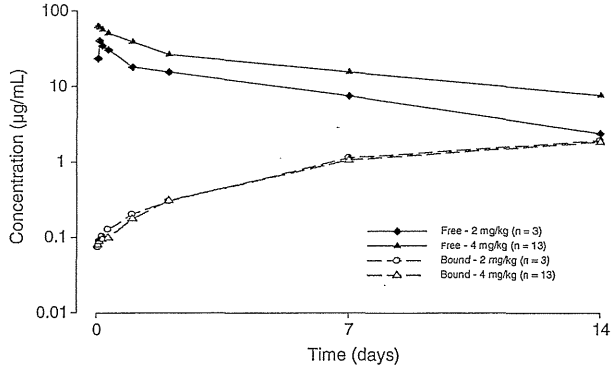

Fig. 1 Mean plasma concentration versus time profiles of free and VEGF-bound aflibercept (log scale) for cycle 1. After aflibercept infusion, the plasma concentration of free aflibercept decreased, whereas that of VEGF-bound aflibercept gradually increased throughout the 2-week cycle. The plasma concentration of free aflibercept increased with the dose, whereas that of VEGF-bound aflibercept was similar at both doses

Table 3 Plasma pharmacokinetic parameters of free and VEGF-bound aflibercept

Mean (CV%) PK parameters of Free and VEGF-bound aflibercept							
Free						Bound	
Dose (mg/kg)	N	C_{max} (μg/mL)	$AUC_{(0-14\ days)}$ (μg·day/mL)	CL (L/day)	$t_{1/2Z}$ (day)	C_{max} (μg/mL)	$AUC_{(0-14\ days)}$ (μg·day/mL)
2	3	41.0 (18)	140 (17)	0.728 (24)	4.95 (28)	1.94 (12)	14.0 (NC)[b]
4	13	72.7 (28)	269 (36)	0.665 (19)[a]	5.59 (14)[a]	1.86 (14)	13.0 (2)[c]

Data are expressed as geometric mean, and percent coefficient of variance is expressed in parentheses.

[a] N=8

[b] N=2, NC = Not Calculable

[c] N=3

the 2.0 and 4.0 mg/kg aflibercept doses were well tolerated by the Japanese patients with mCRC. On the basis of the acceptable overall safety profiles, pharmacokinetic analysis results, and the absence of DLTs, 4.0 mg/kg, administered every 2 weeks, was determined to be the RPTD of aflibercept in combination with FOLFIRI as second-line treatment for the Japanese patients with mCRC. The same dose had been determined as the RPTD in a previous phase I study of aflibercept administered alone and that of the combination of aflibercept with the irinotecan/5-FU/leucovorin (irinotecan/LV5FU2) regimen in non-Japanese patients [20, 21]. A previous multinational phase III study of aflibercept in combination with FOLFIRI had also used the 4.0 mg/kg dose [17]. The maximum tolerated dose could not be determined in the present study, as doses higher than the recommended dose for non-Japanese patients were not tested.

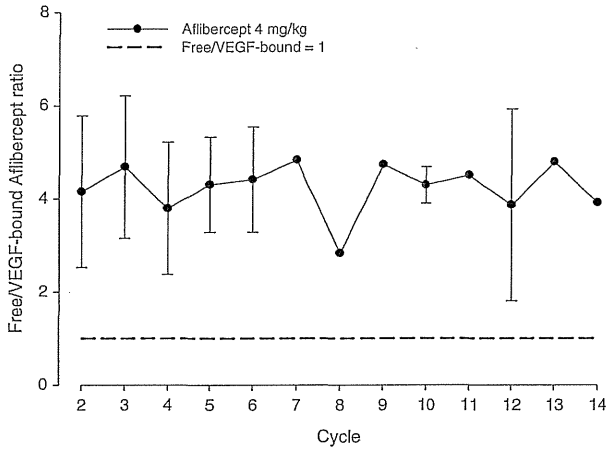

Fig. 2 Ratio of free aflibercept concentration to VEGF-bound aflibercept concentration at the trough (2 weeks after aflibercept administration) across the treatment cycles for dose 2 (4.0 mg/kg). The mean±SD value for each cycle is indicated. Note that the free/VEGF-bound aflibercept concentration ratio was maintained at >1 (dotted line) throughout the treatment duration

The frequently observed adverse events at both doses included those related to bone marrow suppression (eg, neutropenia), gastrointestinal symptoms (eg, diarrhea, constipation, nausea, vomiting, stomatitis, abdominal pain, and decreased appetite), and fatigue. These toxicities are known to be associated with the FOLFIRI backbone regimen. In addition, toxicities known to be associated with VEGF pathway inhibition, such as dysphonia, hypertension, and epistaxis, were also frequently observed.

The mean relative dose intensities for aflibercept were 67 % and 73 % at doses 1 and 2, respectively. The primary reason for the decrease in dose intensities was the treatment cycle delay of >2 d, which occurred in 80 % and 56 % of the cycles at doses 1 and 2, respectively. The most frequent adverse event leading to cycle delay was neutropenia. Although neutropenia was frequently observed and often resulted in cycle delays, the granulocyte colony-stimulating factor was administered to only 2 patients receiving each dose, and all the patients eventually recovered from neutropenia and continued the combination treatment at the initial dose. Of the toxicities reported to be associated with VEGF pathway inhibition, all the events were of grade 2 or lower, except for hypertension. Four patients (30.8 %) receiving dose 2 had grade 3/4 hypertension but could continue the scheduled treatment without aflibercept dose reduction. Proteinuria of grade 2 or lower was observed in 3 patients (23.1 %) receiving dose 2. None of the patients developed any other serious toxicities related to VEGF inhibition, such as arterial or venous thrombotic/thromboembolic events, gastrointestinal perforation, or reversible posterior leukoencephalopathy syndrome (RPLS).

Aflibercept forms inert complexes with VEGF derived from normal and tumor tissues, and these complexes are retained in the systemic circulation. VEGF-bound aflibercept is considered to indicate the amount of endogenous VEGF produced in normal and tumor tissues. Free

aflibercept is available for binding with newly secreted VEGF. The concentration ratio of free aflibercept to VEGF-bound aflibercept at the trough was >1 at dose 2, indicating that the free aflibercept concentration was in excess of that of VEGF-bound aflibercept throughout the treatment duration. Preclinical studies indicated that the biological effects of aflibercept correlated with free aflibercept levels in excess of VEGF-bound aflibercept levels [22]. These pharmacokinetic findings suggest that the 4.0 mg/kg dose provides a sufficient aflibercept concentration to effectively block endogenous VEGF.

The preliminary efficacy analysis in this study showed 1 case (8.3 %) of PR at dose 2. For the 12 patients evaluated, the DCR was relatively high (75.0 %), and the PFS was 7.59 months (95 % CI, 2.33–9.59) at dose 2. Although there is potential for biases owing to the small sample size of this study and heterogeneity in prior line(s) of chemotherapy, our results appear promising in light of the published median PFS (range, 2.5–5.1 months) for FOLFIRI as second-line treatment in patients with mCRC [23–25].

In summary, the RPTD of aflibercept in combination with FOLFIRI for the Japanese patients with mCRC was determined to be 4.0 mg/kg, administered every 2 weeks. The dose was well tolerated and showed favorable pharmacokinetic characteristics and preliminary efficacy. In a recent multinational phase III study evaluating aflibercept plus FOLFIRI versus placebo plus FOLFIRI in patients who had mCRC and previously failed an oxaliplatin-based regimen, the primary end point of overall survival was achieved. The aflibercept plus FOLFIRI combination therapy also showed significant benefits in terms of the secondary end points of PFS and objective response rate. On the basis of the results of this phase III study and our phase I study, further investigation is being planned to evaluate the efficacy and safety of this combination in Japanese mCRC patients.

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Ethical standards This study was approved by the institutional review board of all the participating institutes and was conducted in accordance with the ethical principles of the Declaration of Helsinki and Japanese Good Clinical Practice guidelines.

Conflict of interest Financial support for this study was provided by Sanofi K.K. TY received research grants from Bayer, Taiho, Daiichi-Sankyo, and ImClone; and lecture fees from Chugai, Bristol-Myers Squibb, Yakult, and Merck Serono. KY received lecture fees from Chugai, Bristol-Myers Squibb, and Merck Serono. TF is an employee of Sanofi K.K. The other authors have no conflict of interest.

References

1. Ferrara N, Alitalo K (1999) Clinical applications of angiogenic growth factors and their inhibitors. Nat Med 5:1359–1364
2. Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1:27–31
3. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–1186
4. Gimbrone MA Jr, Leapman SB, Cotran RS et al (1972) Tumor dormancy in vivo by prevention of neovascularization. J Exp Med 136:261–276
5. Folkman J (2002) Role of angiogenesis in tumor growth and metastasis. Semin Oncol 29:15–18
6. Dvorak HF, Dvorak AM, Manseau EJ, Wiberg L, Churchill WH (1979) Fibrin gel investment associated with line 1 and line 10 solid tumor growth, angiogenesis, and fibroplasia in guinea pigs. Role of cellular immunity, myofibroblasts, microvascular damage, and infarction in line 1 tumor regression. J Natl Cancer Inst 62:1459–1472
7. Zhang HT, Craft P, Scott PA et al (1995) Enhancement of tumor growth and vascular density by transfection of vascular endothelial cell growth factor into MCF-7 human breast carcinoma cells. J Natl Cancer Inst 87:213–219
8. Claffey KP, Robinson GS (1996) Regulation of VEGF/VPF expression in tumor cells: consequences for tumor growth and metastasis. Cancer Metastasis Rev 15:165–176
9. Ferrara N, Hillan KJ, Gerber HP, Novotny W (2004) Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 3:391–400
10. Holash J, Davis S, Papadopoulos N et al (2002) VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci U S A 99:11393–11398
11. Presta LG, Chen H, O'Connor SJ et al (1997) Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 57:4593–4599
12. Fischer C, Mazzone M, Jonckx B, Carmeliet P (2008) FLT1 and its ligands VEGFB and PlGF: drug targets for anti-angiogenic therapy? Nat Rev Cancer 8:942–956
13. Fischer C, Jonckx B, Mazzone M et al (2007) Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. Cell 131:463–475
14. Jemal A, Thomas A, Murray T et al (2002) Colorectal cancer incidence, mortality and survival in South-east England between 1972 and 2001: Cancer statistics. CA Cancer J Clin 52:23–47
15. Parkin DM, Bray F, Ferlay J et al (2005) Global cancer statistics. CA Cancer J Clin 55:74–108
16. Saito H (2000) Colorectal cancer screening in Japan. Jpn J Cancer Clin 46:35–42
17. Van Cutsem E, Tabernero J, Lakomy R et al (2012) Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. J Clin Oncol 30:3499–3506
18. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205–216
19. Brookmeyer R, Crowley J (1982) A confidence interval for the median survival time. Biometrics 38:29–41

20. Lockhart AC, Rothenberg ML, Dupont J et al (2010) Phase I study of intravenous vascular endothelial growth factor Trap, aflibercept, in patients with advanced solid tumors. J Clin Oncol 28:207–214
21. Van Cutsem E, Khayat D, Verslype C et al (2012) Phase I dose-escalation study of intravenous aflibercept administered in combination with irinotecan, 5-fluorouracil and leucovorin in patients with advanced solid tumours. Eur J Cancer [Epub ahead of print]
22. Rudge JS, Holash I, Hylton D, Russell M, Jiang S, Leidich R (2007) VEGF Trap complex formation measures production rates of VEGF, providing a biomarker for predicting efficacious angiogenic blockade. Proc Natl Acad Sci U S A 4:18363–18370
23. Tournigand C, André T, Achille E et al (2004) FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: A randomized GERCOR study. J Clin Oncol 22:229–237
24. Muro K, Boku N, Shimada Y et al (2010) Irinotecan plus S-1 (IRIS) versus fluorouracil and folinic acid plus irinotecan (FOLFIRI) as second-line chemotherapy for metastatic colorectal cancer: a randomised phase 2/3 non-inferiority study (FIRIS study). Lancet Oncol 11:853–860
25. Peeters M, Price TJ, Cervantes A et al (2010) Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol 28:4706–4713

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Phase I study of TAS-102 treatment in Japanese patients with advanced solid tumours

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BACKGROUND: TAS-102 consists of α, α, α-trifluorothymidine (TFT) and an inhibitor of thymidine phosphorylase (TPI). We conducted a dose-escalation phase I study in Japanese patients with advanced solid tumours.

METHODS: TAS-102 was administered twice daily on days 1–5 and days 8–12 in a 28-day cycle to patients with solid tumours refractory to standard chemotherapy, to determine its maximum tolerated dose (MTD), dose-limiting toxicities (DLTs), and pharmacokinetics (PKs). MTD was evaluated in cycle 1.

RESULTS: Safety and PKs were evaluated in 21 patients treated with TAS-102 at 30, 40, 50, 60, or 70 mg m^{-2} per day. DLTs, such as grade 4 leucopenia, grade 4 neutropenia, and grade 4 thrombocytopenia, were observed in two patients at doses of 30 and 70 mg m^{-2}. α, α, α-trifluorothymidine and TPI exposures increased dose dependently, and the percentage of decrease in neutrophil count and TFT exposure were significantly correlated. The disease control rate was 50.0% with a median progression-free survival of 2.4 months in 18 colorectal cancer patients. The dose of TAS-102 was not increased above 70 mg m^{-2} per day because of the increased tendency for grade 3 and 4 neutropenia, and 70 mg m^{-2} per day was the recommended dose for phase II studies.

CONCLUSIONS: TAS-102 at 70 mg m^{-2} per day was tolerated in Japanese patients with advanced solid tumours. Phase II studies are ongoing in patients with colorectal cancer.

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Keywords: phase I study; pharmacokinetics; TAS-102; TFT; TPI

α, α, α-trifluorothymidine (TFT), an analogue of thymidine, exhibits two mechanisms of anti-tumour action as follows: inhibition of thymidylate synthase, which is similar to the mechanism of action of 5-fluorouracil (5-FU), and creation of single-strand DNA breaks by incorporating the triphosphate form of TFT into DNA (Fujiwara and Heidelberger, 1970a; Fujiwara *et al*, 1970b). The anti-tumour effects of TFT on colon cancer cell lines and xenograft models refractory to 5-FU (Emura *et al*, 2004b; Temmink *et al*, 2007) are thought to be because of incorporation of TFT into DNA. The reason that TFT is able to affect DNA is that TFT is resistant to DNA glycosylase as compared with 5-FU (Suzuki *et al*, 2011). It is also reported that TFT incorporation into DNA causes instability of DNA (Markley *et al*, 2011). Thus, TFT has been proposed to be effective in patients refractory to 5-FU treatment.

In the initial phase I/II studies of TFT performed in the 1960s, different schedules of intravenous TFT administration were evaluated in chemotherapy-naive patients with metastatic breast cancer and metastatic colorectal cancer. Although early clinical trials showed some anti-tumour activity of TFT, further development of this agent was not undertaken because of inadequate information about the pharmacokinetic (PK) and toxicity profiles (Heidelberger *et al*, 1970; Ansfield and Ramirez, 1971; Emura *et al*, 2004a). On the other hand, concomitant administration of TFT and thymidine phosphorylase inhibitor (TPI; 5-chloro-6- (2-iminopyrrolidin-1-yl) methyl-2, 4 (1*H* 3*H*) -pyrimidinedione hydrochloride) showed an improvement in the PK profile of TFT; thus, the plasma concentrations and anti-tumour activity of TFT increased because of inhibition of TFT degeneration (Fukushima *et al*, 2000). TAS-102 is an oral anti-cancer drug consisting of TFT and TPI combined at a molar ratio of 1 : 0.5. Initial clinical studies of TAS-102 were performed in 111 patients using various dosing schedules.

On the basis of the results of five phase I studies and in consideration of the safety and efficacy, a dose of 50 mg m^{-2} per day was defined as the recommended dose (RD); TAS-102 was given twice a day within 1 h after a meal for 5 days a week for 2 weeks, followed by a 2-week rest (Green *et al*, 2006; Hong *et al*, 2006; Overman *et al*, 2008a; 2008b). Granulocytopenia was consistently identified as a dose-limiting toxicity (DLT).

The primary objective of this phase I study was to establish the maximum tolerated dose (MTD) and DLTs in Japanese patients to determine the optimal phase II dose, and the secondary objective was to examine the PKs and preliminary efficacy of TAS-102.

PATIENTS AND METHODS

Patient population

The eligibility criteria in this study were as follows: (1) Japanese patients with advanced or metastatic solid tumours confirmed by

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Clinical Studies