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A phase I dose-escalation study of aflibercept administered in combination with pemetrexed and cisplatin in patients with advanced solid tumours

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BACKGROUND: To evaluate the safety, pharmacokinetics (PKs), and pharmacodynamics of aflibercept, and to identify the recommended phase II dose (RP2D) of aflibercept in combination with pemetrexed and cisplatin.

METHODS: Aflibercept was administered at escalating doses of 2, 4, or 6 mg kg^{-1} in combination with fixed doses of pemetrexed (500 mg m^{-2}) plus cisplatin (75 mg m^{-2}) every 3 weeks. Blood samples were collected for PK analyses. Serum antiaflibercept antibodies were quantified to assess their impact on systemic aflibercept concentrations.

RESULTS: Eighteen patients were enrolled. One patient dosed at 4 mg kg^{-1} experienced grade 3 hypophosphatemia (dose-limiting toxicity; DLT), which prompted a cohort expansion. No further DLTs were observed in the 4 mg kg^{-1} cohort or the 6 mg kg^{-1} dose cohort. Most common adverse events (AEs) of all grades included (%): fatigue (89), anaemia (89), nausea (83), hyponatremia (78), and neutropenia (72). Grade $\geqslant$3 AEs consistent with anti-vascular endothelial growth factor therapy included (%): hypertension (22), pulmonary embolism (11), and deep vein thrombosis (6). Five patients (28%) experienced mild neurocognitive disturbance. No episodes of reversible posterior leukoencephalopathy syndrome (RPLS) were noted.

CONCLUSION: The results of this phase I study allowed further evaluation of the combination of aflibercept with pemetrexed and cisplatin in a phase II study. The RP2D of aflibercept was 6 mg kg^{-1}, to be administered intravenously every 3 weeks in combination with pemetrexed and cisplatin.

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Vascular endothelial growth factor (VEGF) is a promoter of tumour angiogenesis (Kowanetz and Ferrara, 2006). Vascular endothelial growth factor signals through its receptors (VEGFR) including VEGFR-1 (FLT-1) and VEGFR-2 (FLK 1/KDR), which are expressed in normal and tumour vasculature endothelia. Vascular endothelial growth factor-mediated signalling is thought to be important in the development and progression of multiple solid tumours, and VEGF mRNA and protein overexpression are prognostic of poor outcome (Bonnesen *et al*, 2009; Delli Carpini *et al*, 2010). Thus, the use of VEGF- and VEGFR-targeted agents as cancer therapy has increased dramatically in recent years (Cook and Figg, 2010).

Aflibercept (VEGF Trap; Regeneron Pharmaceuticals, Tarrytown, NY, USA, and Sanofi Oncology, Cambridge, MA, USA) is a recombinant protein consisting of domain 2 from VEGFR-1 fused to domain 3 from VEGFR-2, attached to the hinge region of the Fc(a) domain of human immunoglobulin IgG1. Aflibercept binds all isoforms of VEGF-A, VEGF-B, and placental growth factor. Aflibercept exerts its antiangiogenic effects through regression in the normalisation and remodelling of surviving tumour vessels, and inhibition of neovascularisation (Holash *et al*, 2002).

Previously reported clinical trials have shown that aflibercept has antitumour activity both as a single agent and in combination with chemotherapy (Holash *et al*, 2002; Tang *et al*, 2008; Lockhart *et al*, 2010; Coleman *et al*, 2011; Tabernero *et al*, 2011). The recommended phase II dose (RP2D) is 4 mg kg^{-1} intravenously (i.v.) every 2 weeks when given as a single agent and either 4 mg kg^{-1} administered every 2 weeks or 6 mg kg^{-1} administered every 3 weeks in combination with chemotherapy (Lockhart *et al*, 2010). The most common treatment-related toxicities were consistent with prior studies of anti-VEGF agents, and included proteinuria, hypertension, fatigue, and hoarseness. Combination studies with cytotoxic chemotherapy have shown some increase in chemotherapy-related toxicities (Freyer *et al*, 2008; Limentani *et al*, 2008; Rixe *et al*, 2008; Kuhnowski *et al*, 2010; Novello *et al*, 2011; Tabernero *et al*, 2011).

Pemetrexed in combination with cisplatin is used first-line in the treatment of patients with locally advanced or metastatic non-squamous non-small-cell lung cancer (NSCLC) (Scagliotti *et al*, 2008), and in patients with advanced malignant pleural mesothelioma (MPM) (Vogelzang *et al*, 2003). The addition of a VEGF inhibitor, such as bevacizumab, to chemotherapy has proven to be

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effective in non-squamous NSCLC, with an acceptable toxicity profile (Sandler *et al*, 2006; Spigel *et al*, 2012).

The primary objective of this phase I combination trial was to determine the dose-limiting toxicities (DLTs) and RP2D of aflibercept administered i.v. every 3 weeks in combination with pemetrexed and cisplatin. Secondary objectives were to assess the safety profile of the combination, to characterise the pharmacokinetics (PKs) of aflibercept and pemetrexed, and to evaluate the immunogenicity of aflibercept.

MATERIALS AND METHODS

Patient eligibility

Patients were required to have a histologically confirmed advanced, incurable malignancy that was refractory to conventional therapy, or for which treatment with pemetrexed and/or cisplatin was considered appropriate. Patients had to have measurable disease by Response Evaluation Criteria in Solid Tumours (RECIST) (version 1.1), (Eisenhauer *et al*, 2009) an Eastern Cooperative Oncology Group (ECOG) performance status (PS) $\leqslant 1$, and adequate haematological, hepatic, and renal function. Prior anti-VEGF therapy $\geqslant 4$ weeks from initial administration of aflibercept was allowed. Key exclusion criteria included: (a) prior treatment with aflibercept or pemetrexed; (b) patients whose disease had progressed during cisplatin administration or relapsed within 6 months of completion of cisplatin-based therapy; (c) surgery within the last 28 days; (d) uncontrolled hypertension defined as systolic blood pressure (BP) $\geqslant 150$ mm Hg and/or diastolic pressure $\geqslant 100$ mm Hg (prior antihypertensive medication was allowed); (e) bleeding diathesis or coagulopathy; (f) brain or leptomeningeal metastases (brain imaging was mandatory for study participation).

The study was conducted at two sites and the institutional review board of both participating centres approved the study.

Study design

This was an open-label, dose-escalation phase I trial; three dose levels were planned for aflibercept: (a) 2 mg kg^{-1}, (b) 4 mg kg, and (c) 6 mg kg^{-1}. No intra-patient dose escalation was permitted. In the event patients experienced a DLT at the first dose level, a dose level -1 (aflibercept given at 1 mg kg^{-1}) was planned. Aflibercept, pemetrexed, and cisplatin were administered i.v. on day 1 of each 3-week cycle. Aflibercept was administered over 1 h, followed in sequence by fixed doses of pemetrexed (500 mg m^2 i.v. over 10 min) and cisplatin (75 mg m^2 i.v. administered as per institutional practice; typically 2 h). Patients were supplemented with vitamin B_{12} (1000 mcg intramuscularly) 1 week before the first pemetrexed dose and every three cycles thereafter. A low-dose folic acid preparation or multivitamin with folic acid was administered by mouth daily at doses ranging from 350 to 1000 mcg. This supplementation started at least 5 days before the first dose of pemetrexed, continued throughout the treatment period, and for 30 days after the last dose of pemetrexed. Oral or i.v. dexamethasone (4 mg) was given twice daily the day before, the day of, and the day after pemetrexed administration unless medically contraindicated. Three patients were enrolled in the first-dose level; dose escalation proceeded, following the standard $3+3$ rule, until $>$ one patient experienced a DLT during the first cycle of therapy. The RP2D was then selected as the dose level at which $\leqslant 1$ of 6 patients encountered a DLT during the first cycle of therapy.

Toxicity was graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events version 3.0 (NCI CTCAE v.3.0). Dose-limiting toxicities were defined as adverse events (AEs) attributed as being possibly, probably, or definitely related to the study agents, and fulfilling one of the following criteria: (a) grade 3 or 4 neutropenia complicated by fever $\geqslant 38.5\,^{\circ}$C or infection, or grade 4 neutropenia of at least 7 days duration, (b) grade 3 thrombocytopenia complicated by haemorrhage or grade 4 thrombocytopenia, or (c) any grade 3 or higher non-haematologic toxicity (except fatigue, anorexia, nausea, vomiting, or diarrhoea that was not optimally controlled with appropriate medical intervention). Grade 3 hypertension (BP $\geqslant 150/100$ mm Hg), or BP $\geqslant 180/100$ mm Hg (if the patient had a history of isolated systolic hypertension) that could be controlled within 3 weeks of initiation of oral antihypertensive therapy was not considered a DLT. Likewise, grade 3 proteinuria (>3.5 grams per 24 h) that recovered to <2 grams per 24 h within 3 weeks of onset and/or grade 3 laboratory abnormalities that could reflect tumour burden were not considered as DLTs.

Patient evaluation

Pre-treatment evaluations were performed within 2 weeks of treatment initiation and included history and physical examination, ECOG PS, haematology, serum chemistry, prothrombin time/INR, PTT, and urinalysis. Physical examinations were repeated on day 1 of each subsequent cycle; haematology, chemistry, and urinalysis were measured weekly for the entire study duration.

Baseline radiological investigations were performed within 28 days of treatment initiation. Objective tumour response was assessed by RECIST (version 1.1) every two cycles (Eisenhauer *et al*, 2009). Complete and partial responses (PR) had to be confirmed at least 4 weeks after the initial observation.

Dose modifications

Patients were required to meet the following criteria to receive study drugs on day 1 of a treatment cycle: absolute neutrophil count $\geqslant 1.5 \times 10^9\,l^{-1}$, platelets $\geqslant 100 \times 10^9\,l^{-1}$, creatinine clearance (CrCl) $\geqslant 60$ ml/min, and non-haematologic toxicity recovered to grade $\leqslant 1$.

In the event a DLT occurred during cycle 1 or later, treatment with the triplet regimen was interrupted temporarily. A recovery period of up to 6 weeks was allowed. Patients could resume dosing at a lower dose level upon recovery to grade 2 or better. Recurrence of a drug-related DLT after one dose reduction led to withdrawal of the patient from the study. Patients who required permanent discontinuation from aflibercept were withdrawn from the study. If either cisplatin or pemetrexed was permanently discontinued, the administration of aflibercept and the remaining chemotherapeutic agent was allowed to continue at the same (or lower) dose.

Duration of therapy

Study treatment continued until disease progression, an unacceptable AE, patient's decision to withdraw from the study, or changes in the patient's condition rendering further treatment unacceptable.

Pharmacokinetic analysis

Blood samples were collected to characterise the plasma PK profiles of aflibercept and pemetrexed. For aflibercept, samples were collected on cycle 1 day 1 before aflibercept infusion, at the end of aflibercept infusion, and at 1, 2, 4 and 8 h after completion of pemetrexed infusion; day 2 (24 h), day 8, and day 15. For cycle 2 and beyond, trough samples for aflibercept were taken before aflibercept infusion. For pemetrexed, samples were collected on cycle 1 day 1 before aflibercept infusion, at the end of pemetrexed infusion, and at 0.25, 0.5, 1, 2, and 4 h after completion of pemetrexed infusion, and on day 2 (24 h).

Aflibercept (bound to VEGF or free) in plasma samples was quantified using a validated, direct enzyme-linked immunosorbent assay, with a lower limit of quantification of 15.6 ng ml^{-1} in human plasma for free aflibercept and 43.9 ng ml^{-1} in human plasma for bound aflibercept (Tew *et al*, 2010). To calculate the total aflibercept concentration, the amount of aflibercept present in the bound complex needed to be determined. As 1 ng of complex contains 0.717 ng of aflibercept and 0.283 ng of human VEGF, the concentration of the complex was multiplied by 0.717 to give the adjusted-bound aflibercept concentration (Equation 1).

$$\text{Adjusted bound aflibercept (ng ml}^{-1}) = \text{bound aflibercept (ng ml}^{-1}) \times 0.717 \quad (1)$$

Total aflibercept concentrations were calculated by adding the adjusted-bound aflibercept concentrations to the free aflibercept concentrations at the corresponding time points (Equation 2). Total aflibercept concentrations were only calculated for samples for which both free and bound aflibercept concentrations were available.

$$\text{Total aflibercept (ng ml}^{-1}) = \text{free aflibercept (ng ml}^{-1}) + \text{adjusted bound aflibercept (ng ml}^{-1}) \quad (2)$$

Pemetrexed plasma concentration determination was performed by LC-MS/MS. Briefly, pemetrexed and its internal standard was extracted from a 0.050 ml aliquot of human K_2-EDTA plasma using an automated protein precipitation procedure. The lower limit of quantitation of the assay is 1.00 mcg ml^{-1}. Detection of antiaflibercept antibodies was performed in acid-treated serum samples using an electrochemiluminescence bridging immunoassay (Tew *et al*, 2010).

Observed PK parameters were calculated using non-compartmental analysis (WinNonLin v. 5.3, Pharsight Corporation, Mountain View, CA, USA). Observed half-life ($t_{½}$), clearance (CL), volume of distribution (V_{ss}), maximum plasma concentration (C_{max}), dose-adjusted C_{max} (C_{max}/D), last observed concentration (C_{last}), time of maximum plasma concentration (t_{max}), time of last observed concentration (t_{last}), area under the concentration-time curve (AUC_{inf}), and dose-adjusted AUC_{inf} (AUC_{inf}/D) were calculated for free aflibercept and for pemetrexed. C_{max}, C_{max}/D, C_{last}, t_{max}, and t_{last} were calculated for adjusted-bound aflibercept–VEGF complex. AUC_{inf} was calculated using the log-linear trapezoidal rule.

RESULTS

Patient demographics

Between October 2008 and November 2010, 18 patients were enrolled at the two participating institutions. A median of four cycles of aflibercept (range 1–12) were administered for the entire group. Treatment duration of the 18 patients ranged from 21 days to 315 days (0.7–10 months). At the time of this report, all patients are off study treatment. Table 1 shows their baseline demographics.

Dose escalation and maximum tolerated dose

Four, seven, and seven patients were enrolled in dose levels 1, 2, and 3, respectively. Treatment is summarised in Table 2. Two patients (1 in the 4 mg kg^{-1} cohort and 1 in the 6 mg kg^{-1} cohort) were replaced owing to disease progression before cycle 1 completion (without experiencing a DLT). No cycle 1 DLT was observed at the first dose level (2 mg kg^{-1}). One patient at the 4 mg kg^{-1} dose level experienced treatment-related grade 3 hypophosphatemia (DLT), thus prompting a cohort expansion with an additional three patients. No additional DLT was observed in the expansion cohort allowing for further dose escalation. When aflibercept was dosed at 6 mg kg^{-1} in the next cohort, no DLTs were observed. Thus, aflibercept 6 mg kg^{-1} i.v. every 3 weeks was selected as the RP2D in combination with pemetrexed and cisplatin.

Table 1 Patient characteristics

	Patients ($n = 18$), *n* (%)
Age, years	
Median	61
Range	37–73
Gender	
Male	9 (50)
Female	9 (50)
ECOG PS	
0	2 (11)
1	16 (89)
Type of tumour	
Mesothelioma	5 (28)
Non-small-cell lung cancer	4 (22)
Angiosarcoma	1 (6)
Breast cancer	1 (6)
Cholangiocarcinoma	1 (6)
Endometrial stromal sarcoma	1 (6)
Appendiceal adenocarcinoma	1 (6)
Carcinoma of unknown origin	1 (6)
Rectal cancer	1 (6)
Thyroid poorly differentiated carcinoma	1 (6)
Prior treatment	
Surgery	13 (72)
Chemotherapy	8 (44)
Radiotherapy	7 (39)
No. of prior chemotherapy regimens	
0	10
1	3
2	1
3	2
4	2

Abbreviation: ECOG PS = Eastern Cooperative Oncology Group performance status.

Table 2 Dose level evaluated and DLT encountered

Dose level	Aflibercept dose (mg kg^{-1})	No. of patients treated	No. of patients with DLT	DLT
1	2	4	0	
2	4	7	1	Grade 3 hypophosphatemia
3	6	7	0	

Abbreviation: DLT = dose-limiting toxicity.

Safety and compliance

All 18 treated patients were evaluable for toxicity and experienced at least one AE during the course of the study. The most frequently reported treatment-related AEs are listed in Table 3.

Fatigue (89%) and nausea (83%) were the most frequently reported non-haematologic all grade AEs. Three patients (17%) treated at higher doses of aflibercept had grade 3 fatigue. Nausea (83%), constipation (61%), anorexia (61%), and vomiting (56%)

Table 3 Treatment-related adverse events and laboratory abnormalities

Dose level	Dose level 1		Dose level 2		Dose level 3			
Dose	2 mg kg^{-1}		4 mg kg^{-1}		6 mg kg^{-1}		All	
No. of patients	4		7		7		18	
Grades	All grades	Grades 3/4	All grades	Grades 3/4	All grades	Grades 3/4	All grades (%)	Grades 3/4 (%)
Fatigue	4	0	6	1	6	2	16 (89)	3 (17)
Nausea	4	0	5	0	6	0	15 (83)	0
Constipation	3	0	3	0	5	0	11 (61)	0
Anorexia	3	0	4	0	4	0	11 (61)	0
Vomiting	4	0	3	0	3	0	10 (56)	0
Hypertension	2	1	3	0	5	3	10 (56)	4 (22)
Dysphonia	1	0	3	0	3	0	7 (39)	0
Headache	3	0	1	0	3	0	7 (39)	0
Dizziness	1	0	2	0	4	0	6 (33)	0
Epistaxis	2	0	1	0	3	0	6 (33)	0
Diarrhoea	2	0	2	0	1	0	5 (28)	0
Mucositis	1	0	3	0	1	0	5 (28)	0
Weight loss	0	0	1	0	3	0	4 (22)	0
Memory impairment	1	0	2	0	0	0	3 (17)	0
Arthralgia	0	0	0	0	2	0	2 (11)	0
Pulmonary embolism	0	0	2	2	0	0	2 (11)	2 (11)
DVT	0	0	1	1	0	0	1 (6)	1 (6)
Rash	0	0	0	0	1	0	1 (6)	0
Sensory neuropathy	1	0	0	0	0	0	1 (6)	0
Haematology								
Neutropenia	2	2	4	2	7	2	13 (72)	6 (33)
Thrombocytopenia	2	0	4	0	2	0	8 (44)	0
Anaemia	3	0	7	0	6	0	3 (17)	0
Chemistry								
Hyponatremia	3	1	6	3	5	2	14 (78)	6 (33)
Hypomagnesemia	2	0	4	0	2	0	8 (44)	0
Hypophosphatemia	1	1	2	1	4	1	7 (39)	3 (17)
Hypokalemia	0	0	1	1	1	0	2 (11)	1 (6)
AST (raised)	2	0	5	0	5	0	12 (67)	0
ALT (raised)	2	0	3	0	5	0	10 (56)	0
ALP (raised)	2	0	2	0	3	1	7 (39)	1 (6)
Decreased CrCl	1	0	0	0	0	0	1 (6)	0
Proteinuria	0	0	0	0	1	0	1 (6)	0

Abbreviations: ALP = Alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CrCl = creatinine clearance; DVT = deep venous thrombosis,

were the most relevant gastrointestinal toxicities. These were grade 1 and 2 in all cases, and were managed with supportive care measures. Other toxicities, commonly associated with antiangiogenic therapy, were hypertension (56%), dysphonia (39%), thromboembolic-related events (TREs, 17%), and proteinuria (6%). Hypertension seemed to be more frequent with increasing doses of aflibercept. Four patients (22%) experienced grade 3 hypertension (one patient treated at 2 mg kg^{-1} and three patients treated at 6 mg kg^{-1}), which was managed with oral antihypertensive medication. No patient discontinued study treatment owing to this AE. Dysphonia was mild, and did not lead to dose modification, delay, or treatment discontinuation. Two patients, both treated in the 4 mg kg^{-1} cohort, had grade 4 pulmonary embolism (PE). One patient had a bilateral PE as well as deep vein thrombosis and was withdrawn from the study; the other was asymptomatic and had an incidental unilateral PE documented on a restaging computed tomography scan showing progressive disease (PD). No major haemorrhagic events were observed. One episode of reversible grade 2 proteinuria was observed, in the 6 mg kg^{-1} cohort. It did not require modification of the treatment dose.

Five patients (28%) experienced mild neurocognitive disturbance. These symptoms mostly consisted of a vague sensation of dizziness and mild headache. All episodes were grade 1 in intensity, and mostly observed in female patients. Four patients were treated at dose level 1 (2 mg kg^{-1} of aflibercept) and one patient was at dose level 2 (4 mg kg^{-1} of aflibercept). There was no apparent association with hypertension, or with the cumulative dose of aflibercept. Magnetic resonance imaging (MRI) of the brain was performed in two of these patients and results were normal. The development of these symptoms did not correlate with a specific number of cycles and, after its resolution, patients who were rechallenged did not experience a recurrence of their symptoms. There were no grade 3 or 4 events. These symptoms did not lead to treatment cessation and were reversible in three of the five patients (60%). One patient reported mild cognitive impairment at the end of the study but did not have a formal neurocognitive assessment, and the other patient was lost to follow-up.

A summary of the most common laboratory abnormalities observed throughout the study can be found in Table 3. Grade 3 neutropenia was observed in six patients (33%), leading to dose delay and/or dose reduction of the chemotherapy part of the regimen in the majority of cases. No episodes of febrile neutropenia were documented. Three patients died during the study: two of disease progression and one patient of a pleural effusion, assessed as unrelated to the study treatment.

Dose delays and dose modifications for pemetrexed and cisplatin are provided in Table 4. There were no dose delays or reductions for aflibercept.

Table 4 Dose delays and reductions due to adverse events per dose level and drug

Pt ID	Dose level	Any delay (yes/no)	Main reason of delay	Total length of delay (week)	Any dose reduction			Number/timing of dose reductions
					C	P	A	
001	1	No	NA	NA		No		NA
002	1	Yes	G3 neutropenia	2	Yes	Yes	No	C4D1,
003	1	Yes	G3 neutropenia, G2 thrombocytopenia, G2 CrCl decreased	1	Yes	No	No	C5D1, C7D1
004	1	Yes	G3 nonneutropenic fever	1		No		NA
005	2	No	NA	NA		No		NA
006[a]	2	No	NA	NA		No		NA
007	2	No	NA	NA		No		NA
008	2	Yes	G3 fatigue, G3 neutropenia, G2 thrombocytopenia	1	Yes	Yes	No	C2D1, C3D1
009	2	No	NA	NA		No		NA
010	2	No	NA	NA		No		NA
011	2	Yes	G3 neutropenia, G2 anaemia, decreased CrCl	3	Yes	Yes	No	C2D1, C3D1, C4D1
012	3	Yes	G1 anaemia	2		No		NA
013	3	No	NA	NA		No		NA
014	3	Yes	G3 neutropenia, G2 fatigue, G1 anaemia	3		No		NA
015	3	Yes	G2 pneumonia (non-related)	2		No		NA
016[a]	3	No	NA	NA		No		NA
017	3	Yes	G1 neutropenia, decreased CrCl	1	Yes	No	No	C4D1
018	3	No	NA	NA		No		NA

Abbreviations: A = aflibercept; C = cisplatin; CrCl = creatinine clearance; NA = not applicable; P = pemetrexed. [a]Patient(s) nonevaluable.

Table 5 Pharmacokinetic parameters of pemetrexed, free and adjusted-bound aflibercept in the 2, 4, and 6 mg kg^{-1} cohorts

	Pemetrexed			Free aflibercept			Adjusted-bound aflibercept		
	2 mg kg^{-1} cohort	4 mg kg^{-1} cohort	6 mg kg^{-1} cohort	2 mg kg^{-1} cohort	4 mg kg^{-1} cohort	6 mg kg^{-1} cohort	2 mg kg^{-1} cohort	4 mg kg^{-1} cohort	6 mg kg^{-1} cohort
Number of patients included in the analysis	4	7	7	4	7	7	4	7	7
Mean C_{max} (mg l^{-1})	124 ± 0.02	112 ± 0.07	113 ± 0.05	53.6 ± 7.14	68.6 ± 18.7	148 ± 108	1.73 ± 0.12	2.28 ± 1.03	2.14 ± 0.74
Mean $AUC_{0-21\ d}$ (hr*mg l^{-1})	151 ± 30.1	151 ± 32.7	162 ± 12.5	4824 ± 2316	7920 ± 6024	10608 ± 3648	NC	NC	NC
Mean t_{max} (h)	0.010 ± 0.0	0.010 ± 0.0	0.010 ± 0.0	1.75 ± 1.51	0.912 ± 1.01	0.912 ± 1.01	300 ± 200	386 ± 179	432 ± 132
Mean clearance[a]	56.7 ± 9.73	57.8 ± 14.0	51.7 ± 3.62	0.00764 ± 0.003	0.0111 ± 0.005	0.0111 ± 0.006	NC	NC	NC
Mean $t_{1/2}$ (h)	1.47 ± 0.39	1.63 ± 0.22	1.73 ± 0.37	75.8 ± 13.7	133 ± 130	89.3 ± 25.0	NC	NC	NC

Abbreviations: AUC = area under the curve; C_{max} = maximum plasma concentration; NC = not calculated; t_{max} = time of maximum plasma concentration; $t_{½}$ = observed half-life. The mean ± s.d. is reported. [a]Units of clearance are ml min^{-1} m^{-2} for pemetrexed, and ml min^{-1} kg^{-1} for free aflibercept and adjusted-bound aflibercept.

Activity

Two patients (11%) achieved a confirmed PR, both treated at the 6 mg kg^{-1} cohort. One patient, a 56-year-old male, with a previously untreated NSCLC achieved PR at cycle 6, and discontinued treatment owing to an inflammatory cholangitis (unrelated to study treatment). A 65-year-old male with a chemonaïve mesothelioma achieved a PR at cycle 8, and received 12 cycles of therapy, until PD was documented. Eleven (61%) patients had SD as their best response.

Pharmacokinetic analysis

The PK parameters for unbound (free) and adjusted-bound aflibercept are shown in Table 5. The mean C_{max} of free aflibercept increased in a dose-proportional manner when comparing the 2, 4, and 6 mg kg^{-1} cohorts. The concentration–time profiles of free aflibercept are characterised by a consistent $t_{½}$ over the dosing interval at all three dose levels (Figure 1). The mean CL and V_{ss} of free aflibercept did not change over the 2–6 mg kg^{-1} dose range, suggesting target (endogenous VEGF) saturation. The mean dose-adjusted AUC during the first dosing interval ($AUC_{0\text{-}21\ day}$/dose) of free aflibercept was dose-proportional at the two higher dose levels of 4 and 6 mg kg^{-1}, indicating near-target saturation in the systemic circulation after one dose. Following i.v. administration, free aflibercept binds endogenous VEGF to form a monomeric aflibercept–VEGF complex. The complex reaches a plateau, and the concentration of adjusted-bound complex remains constant with repeat dosing of aflibercept at 2, 4, and 6 mg kg^{-1} over the 21-day dosing interval. Bound aflibercept concentrations were comparable at all three dose levels, suggesting saturation of endogenous VEGF binding (Figure 1). The cohorts differed only in their time-to-plateau, which was longest for the 2 mg kg^{-1} cohort and shorter for the 4 and 6 mg kg^{-1} cohorts.

Pemetrexed PK parameters were calculated to assess the effect, if any, of aflibercept administration on systemic pemetrexed concentrations (Table 5). The systemic concentration of and exposure to pemetrexed were not altered by concomitant administration of aflibercept. In particular, the kinetics of pemetrexed were linear over the first dosing interval, consistent with literature values of pemetrexed PK parameters when pemetrexed is administered with cisplatin in the same study

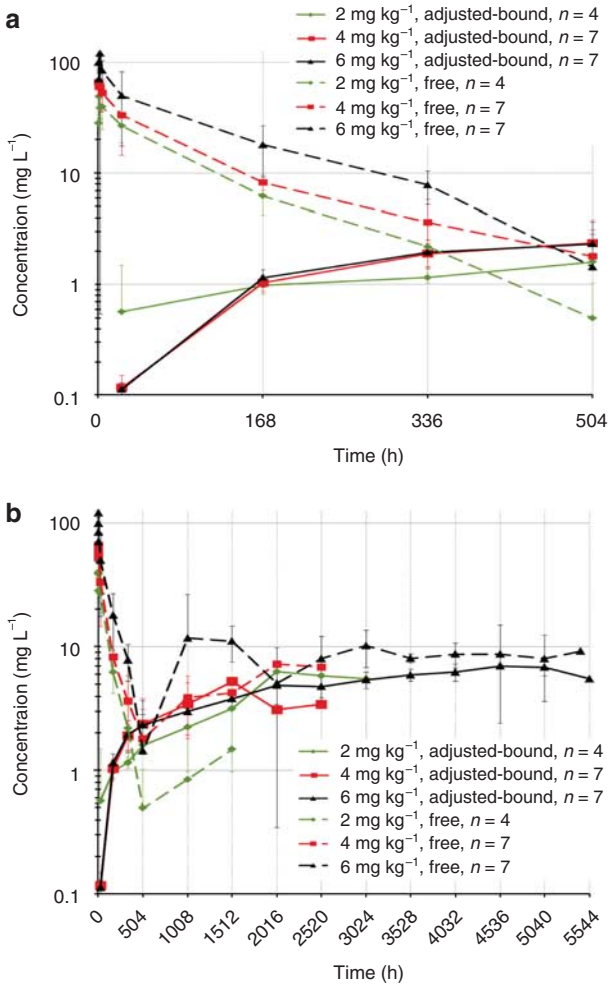

Figure 1 (**A**) Mean aflibercept (free and adjusted-bound) concentration–time profiles after first, single i.v. administration of aflibercept. (**B**) Mean aflibercept (free and adjusted-bound) concentration–time profiles after i.v. administration of multiple doses of aflibercept.

population composition as this study (Dickgreber *et al*, 2009). The pemetrexed C_{max}, AUC, t_{max}, and $t_{½}$ were not statistically different ($P<0.05$) between the 2, 4, and 6 mg kg^{-1} aflibercept cohorts. In addition, the mean plasma concentration of pemetrexed at any given time point was the same regardless of aflibercept dose. Further, free and bound aflibercept drug concentrations in combination with pemetrexed are comparable to those observed with aflibercept monotherapy (Tew *et al*, 2010), suggesting that aflibercept PK are not affected by concomitant pemetrexed administration.

No antiaflibercept antibodies were detected in analysed samples.

CONCLUSION

This study has evaluated the feasibility of the combination of i.v. aflibercept in conjunction with cisplatin and pemetrexed in patients with advanced solid tumours. The RP2D of aflibercept was determined to be 6 mg kg^{-1} i.v. every 3 weeks. The most common treatment-related clinical AEs were fatigue and nausea. Fatigue was observed more frequently than in prior studies with cisplatin and pemetrexed combinations both in NSCLC and MPM patients (Shepherd *et al*, 2001; Vogelzang *et al*, 2003; Scagliotti *et al*, 2008). The most frequently reported haematologic toxicities (i.e., neutropenia and thrombocytopenia) do not appear to differ significantly from previous studies (Vogelzang *et al*, 2003; Kim *et al*, 2008; Scagliotti *et al*, 2008).

Anti-VEGF therapy has been associated with several side effects common to this class of agents. The incidence of vascular-related toxicities (i.e., hypertension, proteinuria, thrombosis, and haemorrhage) in this study was generally similar to that previously reported in prior studies of anti-VEGF therapy (Kuenen *et al*, 2002), including aflibercept with chemotherapy (Freyer *et al*, 2008; Limentani *et al*, 2008; Rixe *et al*, 2008; Kuhnowski *et al*, 2010). Prior reports have indicated a potential negative interaction between VEGF modulators and cisplatin (Kuenen *et al*, 2002; Marx *et al*, 2002). As VEGF has an important role in endothelial cell homeostasis and modulates the production of nitric oxide, deprivation of VEGF can potentially lead to a shift in endothelial cells to a prothrombotic state (Li and Keller, 2000). Chemotherapeutic agents, especially cisplatin, can induce activation of platelets, monocytes, and endothelial cells (Togna *et al*, 2000); as such, further exacerbation of hypercoagulability could potentially occur, resulting in a high rate of thromboembolic events. Two patients (11%) experienced three TREs in this study (two episodes of PE and one episode of DVT) deemed to be at least possibly related to the study treatment. The reported incidence of TREs in prior studies of aflibercept and chemotherapy ranges from 3 to 22% (Tabernero *et al*, 2012). In addition, a meta-analysis of anti-VEGF class AEs in three placebo-controlled phase III trials with aflibercept demonstrated that venous thrombotic events were not increased with aflibercept (Tabernero *et al*, 2012). The incidence of TREs in unselected patient populations with NSCLC and/or mesothelioma is 3–31% (Chew *et al*, 2008). It is therefore difficult to ascertain whether the addition of aflibercept to the cisplatin–pemetrexed combination actually poses a higher risk of developing TREs.

Vascular endothelial growth factor has an important role in regulating glomerular permeability (Kanellis *et al*, 2004). Thus, the inhibition of VEGF in the kidney glomeruli can result in proteinuria. Several clinical trials of aflibercept have confirmed that proteinuria is a potential toxicity, with an overall incidence of grade 3/4 up to 8% (Tabernero *et al*, 2012). Nephrotoxicity is one of the major side effects of cisplatin, which occurs in a dose-dependent manner in 25–30% of patients receiving a single dose of cisplatin. Renal toxicity is also accumulative. Glomerular injury is one of the potential mechanisms of nephrotoxicity when cisplatin is used (Sanchez-Gonzalez *et al*, 2011). Although glomerular injury occurs less frequently (because it is associated with high drug exposures) than tubular toxicity, vascular or interstitial damage, it may lead to proteinuria. Cisplatin-induced glomerular injury is characterised by a marked fall in the glomerular filtration rate. In this study, the frequencies of decreased CrCl and of proteinuria were both low ($n=1$ each, 6%) and no grade $\geqslant 3$ of such events were observed (Table 3).

In this study, we observed an increased rate of neurological symptoms, particularly in the form of a poorly defined, mild neurocognitive disturbance, often described by patients as dizziness or lightheadedness. There was no apparent dose dependence, as four episodes of this neurocognitive disturbance were documented when aflibercept was administered at 2 mg kg^{-1}, and only one patient reported it in the 4 mg kg^{-1} cohort. Evaluation of these vague neurological symptoms steered towards the consideration of RPLS, which has been associated with the administration of anti-VEGF agents and/or cisplatin (Ito *et al*, 1998; Marinella and Markert, 2009; Leighl *et al*, 2010). However, differences between the symptoms observed in this patient subgroup and the most commonly reported characteristics of RPLS were noted. First, most cases of RPLS reported in the literature have been associated with severe and often acute hypertension; this was not the case in our study. All patients had their BP monitored on a weekly basis. For those who developed these mild neurocognitive symptoms, no significant changes were

observed between BP reading before and after the development of symptoms (data not shown). Endothelial dysfunction has been implicated in the pathophysiology of RPLS, and has been observed in other, nontumoral, clinical settings, such as chronic renal failure, nephritis, haemolytic uraemic syndrome, and/or metabolic disturbances (e.g., hypomagnesemia) (Hinchey *et al*, 1996). Therefore, we further investigated if the development of this cognitive disorder was associated with decreased CrCl, the onset of proteinuria, or the presence of electrolyte abnormalities. All patients who developed neurocognitive symptoms had a normal CrCl at baseline, and no changes were observed in association with the onset of symptoms (data not shown). Second, other potentially serious neurological symptoms (e.g., alteration in mental status, hallucinations, and seizures) are usually present in the development of RPLS. These symptoms were not observed in patients in our study though reports of mild headache and memory impairment were noted in several patients. Finally, abnormalities in MRI, including vasogenic oedema, have been described consistently and are key to diagnosis in patients affected by RPLS (Hinchey *et al*, 1996). In this phase I study, in those patients who had MRI scans ($n = 2$) after developing neurocognitive symptoms, no MRI abnormalities were noted.

Pharmacokinetic analysis showed that trough concentrations of free (unbound) aflibercept were higher than adjusted-bound aflibercept trough concentrations in patients receiving either 4 or 6 mg kg^{-1} aflibercept. Maintenance of systemic free aflibercept concentration above the adjusted-bound aflibercept trough concentration ensures maintenance of target saturation. As target saturation is thought to parallel clinical efficacy, PK analysis suggests that a dose of 6 mg kg^{-1} i.v. every 3 weeks has the best chance of clinical efficacy via maintenance of target saturation over the entire dosing interval. With respect to the potential impact of aflibercept administration on pemetrexed concentrations, the systemic concentration of and exposure to pemetrexed were not altered by concomitant administration of aflibercept.

In summary, this study showed that the administration of aflibercept 6 mg kg^{-1} i.v. every 3 weeks in combination with cisplatin and pemetrexed led to a slightly higher than expected rate of the side effects (mainly increased fatigue) than that observed with the chemotherapy regimen alone. The mild neurocognitive disturbances seen in five patients were investigated and did not suggest RPLS at this stage, but led to a higher level of observation for this toxicity in the phase II study. The phase II study of this regimen was conducted in previously untreated nonsquamous NSCLC (clinicaltrials.gov identifier: NCT00794417) and results will be reported separately, but it is noted that the study was discontinued early owing to a higher than anticipated incidence of RPLS.

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Conflict of interest

LLS and HAW receive research funding from Regeneron Pharmaceuticals. FAS has received honoraria from Regeneron Pharmaceuticals. AT, LL, JL, BG, and EBL are employees of Regeneron Pharmaceuticals. All remaining authors declare no conflict of interest.

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