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PHASE I STUDIES

A phase I and pharmacokinetic study of afilbercept with FOLFIRI: comparison of Chinese and Caucasian populations

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Summary *Background* This study assessed the preliminary safety, pharmacokinetics (PK) and anti-tumor effects of aflibercept in combination with 5-fluorouracil, leucovorin and irinotecan (FOLFIRI) in Chinese patients with previously-treated advanced solid malignancies. *Patients and Methods* This open-label single-arm Phase I study conducted at two centers in China included adult (≥18 years) patients with metastatic or unresectable solid malignancies who had received ≥1 prior treatment. Patients received aflibercept 4 mg/kg IV on Day 1 followed by FOLFIRI over Days 1 and 2 every 2 weeks, and were assessed for safety, tumor response, PK parameters and immunogenicity. Post-hoc analyses included calculation of progression-free survival (PFS) for patients with colorectal cancer (CRC). *Results* A total of 20 patients were enrolled. The most common Grade 3/4 adverse events included neutropenia (35%), hypertension (30%), stomatitis (20%) and proteinuria (20%), and no anti-aflibercept antibodies were detected. Six patients achieved a partial response, and in 15 patients with CRC median PFS was 5.95 months (95% CI: 5.29–8.77). Free aflibercept remained in excess of VEGF-bound aflibercept for the majority of the study treatment duration. The mean free aflibercept values for C_{max} (64.8 μg/mL) AUC (291 μg.day/mL), CL (0.92 L/day) and V_{ss} (5.9 L) were similar to those measured in Caucasian patients. The addition of aflibercept did not influence the PK of the chemotherapy agents. *Conclusion* For Chinese patients with pre-treated advanced solid malignancies, 4 mg/kg of aflibercept in combination with FOLFIRI was well-tolerated, demonstrated preliminary anti-tumor activity and had a PK profile consistent with that in Caucasian patients.

Keywords Aflibercept · Safety · Phase I study · Pharmacokinetics · Solid tumors · Chinese patients

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Introduction

Many types of cancer are dependent on angiogenesis, the formation of new blood vessels from existing vasculature, to establish a source of nutrition and oxygen, support growth and enable metastasis [1, 2]. Vascular endothelial growth factor (VEGF) is a cytokine that is critical to angiogenesis [3]. Overexpression of VEGF has been associated with increased tumor vascularity, proliferation, progression, invasion, metastasis and poor prognosis [4–6]. Given the importance of angiogenesis to tumor growth and development, VEGF has become a major therapeutic target for cancer [4, 7].

Current standard treatments for metastatic colorectal cancer (mCRC) have evolved to include the addition of targeted

biologic therapies to the combination of 5-fluorouracil (5-FU) and leucovorin with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI). Targets for such biologic therapies include VEGF (such as bevacizumab) and epidermal growth factor receptor (EGFR) (such as cetuximab and panitumumab) [8, 9], and these therapeutic regimens have demonstrated clinically significant improvements in outcomes such as overall survival (OS) and progression-free survival (PFS) in mCRC [10].

Aflibercept is a recombinant human fusion protein that binds to VEGF-A and -B and placental growth factor growth to prevent interaction with VEGR receptors, and has been shown to inhibit tumor growth, tumor vessel density and metastatic spread in vivo [11–13]. Early clinical studies established the pharmacokinetic (PK) profile of aflibercept in Caucasian (TCD6118) and Japanese (NCT00921661) patients [14–16]. In addition, these early studies provided preliminary safety data and showed the anti-tumor activity of aflibercept as a single agent and in combination with chemotherapy regimens including 5-FU, irinotecan and leucovorin in the treatment of solid malignancies [14–16]. The Phase III international, double-blind, comparative VELOUR study, demonstrated that, compared with a combination of 5-FU, leucovorin and irinotecan alone (FOLFIRI regimen), aflibercept in combination with FOLFIRI in pre-treated patients with mCRC significantly improved median OS (13.5 vs. 12.1 months, $p = 0.0032$); PFS (6.9 vs. 4.7 months, $p < 0.0001$) and overall response rate (19.8% vs. 11.1%, $p = 0.0001$) [17].

Based on data from the pivotal clinical trial, aflibercept has been approved in 65 countries including the US and the EU (as of January 1, 2016) for use in combination with FOLFIRI as a second-line treatment for mCRC, and has been incorporated into international treatment guidelines as one of the recommended therapies for patients who fail first-line treatment with an oxaliplatin-based regimen [18, 19]. However, the aflibercept clinical development program did not include Chinese patients, and the drug is not yet approved in China. A separate Phase III study (AFLAME NCT01661270), was established to investigate aflibercept and FOLFIRI as second-line treatment in East-Asian patients with mCRC. The outcomes of AFLAME will provide further information on the safety and efficacy profile of aflibercept with FOLFIRI in Chinese and other East-Asian patients with previously-treated mCRC.

Given the lack of aflibercept data in Chinese patients, the objectives of this study were to assess the preliminary safety, PK, immunogenicity, and anti-tumor effects of the intravenous (IV) aflibercept dose (4 mg/kg) established in prior Phase I-III studies, [14–17, 20–22] in combination with FOLFIRI in Chinese patients with pre-treated, advanced solid malignancies.

Methods

Study design and patient eligibility

This was an open-label, single-arm Phase I study conducted at two study centers in mainland China. The study included adults (≥18 years) with histologically- or cytologically-confirmed metastatic or unresectable solid malignancies who had received at least one prior treatment and for whom FOLFIRI treatment was appropriate. Eligible patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) ≤1 and adequate liver, renal and bone marrow function.

Key exclusion criteria included prior treatment with chemotherapy, hormonal therapy, radiotherapy, surgery or an investigational agent within 4 weeks (6 weeks for nitrosourea agents, mitomycin C or immunotherapy) of study enrollment, squamous cell lung cancer, history of central nervous system involvement, clinically significant cardiac disease or myocardial infarction within 6 months before study entry, uncontrolled hypertension of >150/100 mmHg, gastrointestinal (GI) disorders including hemorrhage, perforation or inflammatory conditions within the last 3 months, unresolved bowel obstruction, evidence of clinically-significant bleeding diathesis or underlying coagulopathy and pregnant or breast-feeding women.

The study consisted of a 21-day baseline period, a treatment period (2 weeks per cycle), and a post-treatment follow-up period of 90 days after the last study treatment administration. The study protocol was approved by local ethics committees and all patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and was registered at Clinicaltrials.gov (NCT01930552).

Study treatment

Eligible patients received 4 mg/kg of aflibercept by IV infusion over 1 h on Day (D) 1 every 2 weeks, immediately followed by FOLFIRI: irinotecan 180 mg/m^2 IV infusion for 90 min, together with leucovorin 400 mg/m^2 by IV infusion for 2 h, followed by 5-FU 400 mg/m^2 by IV bolus over 2–4 min then 5-FU 2400 mg/m^2 continuous IV infusion over 46 h starting on D1. Anti-emetic premedication with a serotonin 5-HT$_3$ receptor antagonist, with or without dexamethasone 10 or 20 mg by IV or equivalent, was administered prior to aflibercept.

Safety and anti-tumor assessments

Safety was assessed through the recording of adverse events (AEs) and collection of laboratory data (hematology, clinical chemistry and urinalysis), vital signs and by

electrocardiogram (ECG). Clinical and laboratory AEs were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 3.0.

Disease status was assessed by computed tomography (CT) or magnetic resonance imaging (MRI) at Baseline, every 6 weeks, whenever disease progression was suspected, and at the end of study treatment. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

Pharmacokinetics

Blood samples (4.5 mL) for both free and VEGF-bound aflibercept were obtained during Cycle 1 pre-treatment, before the aflibercept end of infusion (EOI, 1 h) then at 1, 3, 7 (D1), 23, 29 (D2), 47 (D3), 167 (D7) and 335 h (D14) after the end of aflibercept infusion, pre-dose on D1 of every odd-numbered cycle, and then at 30 and 90 days after the last administration of aflibercept. Plasma concentrations were measured by validated enzyme-linked immunosorbent assays (ELISA). Concentrations of VEGF-bound aflibercept were expressed as free aflibercept equivalents by adjusting the measured complex levels for the proportion of the molecular weight of the complex before PK analysis. The corresponding lower-limits of quantification (LLOQ) for free and adjusted VEGF-bound aflibercept were 15.6 ng/mL and 31.5 ng/mL, respectively.

Blood samples for testing of irinotecan and SN-38 concentration were obtained before aflibercept infusion, just before EOI of irinotecan (1.5 h), then at 2, 4 and 23 h after the initiation of irinotecan infusion during Cycle 1 (LLOQ 10.0 ng/mL and 1.0 ng/mL, respectively). 5-FU concentration was measured before the start of aflibercept infusion, and at 3, 21 and 45 h, during IV 5-FU infusion for, Cycle 1 (LLOQ =5.0 ng/mL).

All PK parameters were calculated by non-compartmental analysis (PKDMS Version 2 running with WinNonLin Professional, Version 5.2.1, PharSight, Raleigh-Durham, NC, USA). The PK parameters for free and VEGF-bound aflibercept were maximum (C_{max}) and trough (C_{trough}) plasma concentrations, time to reach C_{max} (t_{max}), time to the last concentration observed above LLOQ (t_{last}), area under the curve until last quantifiable time point (AUC_{last}) and over the dosing interval ($AUC_{0–14\ day}$). AUC extrapolated to infinity (AUC), total body clearance (CL), volume of distribution at steady

Table 1 Patient demographics and baseline characteristics

Characteristic	All patients (n = 20)
Median age, years (range)	52.0 (26–70)
Male, n (%)	13 (65)
Median weight, kg (range)	63.5 (46–78.5)
ECOG PS, n (%)	
0	0
1	20 (100)
Primary tumor site, n (%)	
Esophagus	2 (10)
Gastric and EGJ	2 (10)
Colorectal	15 (75)
Peritoneum	1 (5)
Prior radiation therapy, n (%)	2 (10)
Prior surgery, n (%)	14 (70)
Prior anticancer treatment, n (%)	
Chemotherapy	20 (100)
Biologics	2 (10)
Median number of prior anticancer treatments[a] (range)	1 (1–3)

ECOG PS: Eastern Cooperative Oncology Group performance status, *EGJ*: esophageal-gastric junction

[a] Excluding radiation therapy and surgery

Table 2 Safety summary

	All Grades, n (%)	Grade 3/4, n (%)
Patients with any AE	20 (100)	14 (70)
Non-hematological AEs[a]		
Decreased appetite	18 (90)	0
Nausea	17 (85)	0
Vomiting	14 (70)	1 (5)
Hypertension	14 (70)	6 (30)
Asthenia	9 (45)	0
Diarrhea	9 (45)	3 (15)
Abdominal pain	8 (40)	1 (5)
Stomatitis	8 (40)	4 (20)
Proteinuria	7 (35)	4 (20)
Epistaxis	7 (35)	0
Upper respiratory tract infection	5 (25)	0
Dizziness	5 (25)	0
Fatigue	4 (20)	0
Pyrexia	3 (15)	0
Dysphonia	3 (15)	0
Constipation	3 (15)	0
Alopecia	3 (15)	0
Hypokalemia	3 (15)	1 (5)
Hematological AEs[a]		
Leukopenia	11 (55)	3 (15)
Neutropenia	10 (50)	7 (35)
Febrile neutropenia	3 (15)	3 (15)
Thrombocytopenia	3 (15)	1 (5)

AE: adverse event

[a] Events listed are All Grade AEs reported in ≥15% patients

Table 3 Summary of best overall confirmed response

Primary tumor site (n = 20)	Best overall confirmed response			
	PR	SD	PD	Not evaluable
Colorectal (n = 15)	4	9	1	1
Esophagus (n = 2)	1	1		
Gastric and EGJ (n = 2)	1	1		
Peritoneum (n = 1)		1		

EGJ: esophageal-gastric junction, *PD*: progressive disease, *PR*: partial response, *SD*: stable disease

state calculated after single IV administration (V_{ss}) and terminal elimination half-life ($t_{1/2z}$) were also estimated for free aflibercept. For irinotecan and SN-38, the parameters were C_{max}, t_{max}, AUC_{last}, AUC, $t_{1/2z}$ and metabolic ratio based on molecular weight (R_{met}). CL and V_{ss} were estimated for irinotecan only. For 5-FU, steady state concentration during constant rate infusion (C_{ss}) and clearance at steady state (CL_{ss}) were estimated.

Immunogenicity

Blood samples for anti-aflibercept antibody levels were collected pre-dose on D1 of every odd-numbered cycle, then at 30 and 90 days after the last dose of aflibercept. The presence of anti-aflibercept antibodies was evaluated in serum using a non-quantitative assay, and a titer-based bridging immunoassay.

Statistical methods

Twenty eligible patients were planned to be treated in this study. The anti-tumor and safety-related analyses were performed in an all-treated population, which included patients who took at least one dose (including partial dose) of study treatment, i.e. aflibercept or FOLFIRI, regardless of correct/incorrect dose assignment.

The safety analysis used descriptive statistics to summarize treatment-emergent AEs (TEAEs) and laboratory values of hematology and biochemistry endpoints. TEAEs were reported from the first dose of study medication to 30 days after the last dose of study medication. The PK analysis population included all patients who received at least 1 cycle of aflibercept and for whom the primary PK data were considered sufficient and interpretable.

Plasma concentrations and PK parameters of free and VEGF-bound aflibercept, irinotecan and its active metabolite SN-38, and 5-FU were calculated and summarized using descriptive statistics including mean, standard deviation (SD), coefficient of variation (CV) and median (minimum–maximum) values for applicable PK parameters, respectively. Free aflibercept and VEGF-bound aflibercept C_{trough} and their ratio were summarized using the same descriptive statistics as above for each odd-numbered cycle and presented graphically using mean and SD for steady-state assessment. Post-hoc exploratory analyses were also conducted to compare the PK parameters observed at Cycle 1 between treatment responders and non-responders in the PK analysis population and to assess the PFS of the CRC patients.

Results

Patient characteristics

Between September 2013 and February 2014, 20 Chinese patients were enrolled at two sites in China. All patients completed at least one cycle of study treatment and PK sample collection, and entered the analysis population. The baseline

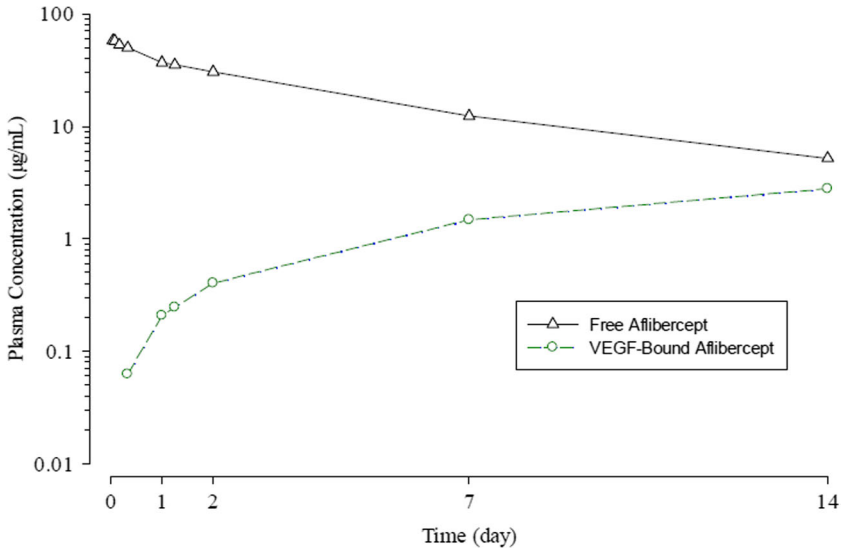

Fig. 1 Mean plasma concentration-time profiles of free and VEGF-bound aflibercept at Cycle 1

characteristics of the patients are presented in Table 1. Overall, 65% of patients were male, the median age was 52 years and all had an ECOG PS of 1 at study entry. All patients had received prior chemotherapy with a median of 1 (range 1–3) prior anticancer regimens. Of the 15 CRC patients, one had prior exposure to bevacizumab and one to cetuximab.

Safety and tolerability

Patients received a total of 193 cycles of study treatment with a median of 9 (range 2–20) cycles administered per patient. All patients had discontinued study treatment at the time of final database lock (January 2015). Twelve patients discontinued study treatment permanently due to disease progression, seven patients at their own request, and one patient due to an AE (anastomotic stenosis).

All patients experienced at least one TEAE. The most frequently-reported (≥15% of patients) are presented in Table 2 and include GI toxicities, hematological abnormalities, hypertension, proteinuria and asthenia. The most common Grade 3/4 TEAEs were neutropenia, leukopenia, febrile neutropenia, hypertension, proteinuria, stomatitis and diarrhea. TEAEs of special interest were hypertension (70%), hemorrhagic events (35%), impaired wound healing (10%) and venous thrombosis (5%).

There were no deaths during the study period. Three patients each experienced one serious TEAE; hemorrhoids, venous thrombosis in the limb of a patient with a history of deep vein thrombosis and anastomotic stenosis (considered unrelated to study treatment but leading to permanent discontinuation of study treatment). Proteinuria was observed in 13 patients (65.0%), four of whom were classified as Grade 3. No

Table 4 Plasma pharmacokinetic parameters of free and VEGF-bound aflibercept in Chinese (TCD11470) and Caucasian (TCD6118) patients

PK parameters	Chinese patients (TCD11470)[a,b]		Caucasian patients (TCD6118)[a,b]		Chinese/Caucasian Geometric Mean Ratio	
	Plasma free aflibercept (n = 20)	Plasma VEGF-bound aflibercept (n = 20)	Plasma free aflibercept (n = 11)	Plasma VEGF-bound aflibercept (n = 11)	Plasma free aflibercept	Plasma VEGF-bound aflibercept
t_{max} (day)[c]	0.04 (0.04–0.17)	14.01 (12.88–15.94)[d]				
C_{max} (μg/mL)	64.8 ± 16.9 (63.1) [26]	2.78 ± 0.625 (2.72) [22][d]	67.8 (64.9) [31]	1.97 (1.93) [21]	0.97	1.41
t_{last} (day)[c]	14.02 (12.88–19.98)	14.01 (12.88–15.94)[d]				
AUC_{last} (μg.day/mL)	252 ± 49.5 (248) [20]	20.8 ± 5.81 (20.1) [28][d]		16.1(15.8) [21]		1.28
AUC (μg.day/mL)	291 ± 55.0 (286) [19][e]		311 (296) [30][f]		0.97	
$AUC_{0–14\ day}$ (μg.day/mL)	249 ± 44.7 (244) [18]	19.8 ± 6.11 (19.0) [31][g]		14.3 (14.0) [21][h]		1.35
CL (L/day)	0.915 ± 0.249 (0.886) [27][e]		0.964 (0.921) [32][f]		0.96	
CL/Weight (L/day/kg)	0.0143 ± 0.00304 (0.0140) [21][e]		0.0142 (0.0134) [39][f]		1.05	
V_{ss} (L)	5.87 ± 1.82 (5.62) [31][e]		6.35 (5.75) [45][f]		0.98	
V_{ss}/Weight (L/kg)	0.0915 ± 0.0230 (0.0889) [25][e]		0.0932 (0.0834) [44][f]		1.07	
$t_{1/2z}$ (day)	4.97 ± 0.644 (4.93) [13][e]					

AUC: area under the curve extrapolated to infinity, $AUC_{0–14\ day}$: area under the curve over the dosing interval, AUC_{last}: area under the curve until last quantifiable time point, *CL*: total body clearance, *CL/Weight*: CL normalized by weight, C_{max}: maximum plasma concentration, *PK*: pharmacokinetics, $t_{1/2z}$: terminal elimination half-life, t_{max}: time to reach maximum plasma concentration, t_{last}: time corresponding to the last observed concentration above the lower limit of quantification, *Vss*: volume of distribution at steady state after a single dose, V_{ss}/*Weight*: V_{ss} normalized by weight

[a] Selected PK parameters included from TCD6118 based on data availability

[b] Continuous variables are summarized as mean ± SD (geometric mean) [coefficient of variation %] unless otherwise stated

[c] Median [range]

[d] n = 18, 2 patients with t_{last} outside the 2-week dosing interval

[e] n = 19, one patient not included in calculation

[f] n = 9

[g] n = 13, parameter not calculable for 7 patients

[h] n = 5

unexpected significant changes in vital signs, ECG or physical findings were observed.

Immunogenicity

No anti-aflibercept antibodies were detected in any of the patients evaluable for immunogenicity (19 out of 20 treated).

Anti-tumor activity

All of the 20 patients who received treatment were evaluable for tumor response (Table 3). A total of six confirmed partial responses were observed in this pre-treated patient population: four out of the 15 patients with CRC, one out of the two patients with esophageal cancer and one out of the two patients with gastric and esophageal-gastric junction (EGJ) cancer. In a post-hoc exploratory analysis, the median PFS of the 15 CRC patients was 5.95 months (95% CI: 5.29–8.77).

Pharmacokinetics

The mean concentration–time profiles of free and VEGF-bound aflibercept are presented in Fig. 1. The PK parameters of free and VEGF-bound aflibercept at Cycle 1 are presented in Table 4. Following a first administration of aflibercept, the mean (CV%) C_{max} and $AUC_{0-14\ day}$ were 64.8 μg/mL (26%) and 249 μg.day/mL (18%), respectively for free aflibercept and 2.78 μg/mL (22%) and 19.8 μg.day/mL (31%), respectively for VEGF-bound aflibercept.

Median t_{max} was observed at the end of infusion (median t_{max} = 1 h) for free aflibercept while VEGF-bound aflibercept concentrations increased regularly over the first cycle and reached C_{max} at the end of Cycle 1 (median t_{max} = 14 days). The $t_{1/2z}$ of free aflibercept was approximately 5 days, associated with a clearance of 0.9 L/day and a volume of distribution of approximately 6 L. The total variability of both free and VEGF-bound aflibercept PK parameters was low to moderate (13–31%). The PK parameters of free and VEGF-bound aflibercept at Cycle 1 for treatment responders and non-responders are presented in Table 5.

Trough concentration showed high total variability over treatment cycles (CV range: 25–78%) and did not allow an accurate assessment of steady state (Table 6). The mean free-to-VEGF-bound aflibercept C_{trough} ratio was roughly constant from Cycles 3 to 9, showing a slow decrease and reaching a minimal value at Cycle 5 and again at Cycle 11 (mean [CV%]: 1.25 [52%] μg/mL and 1.25 [not calculated] μg/mL, respectively). The ratio remained >1 for Cycles 2 to 9, reducing to 0.988 for Cycle 11 (n = 2) signifying that free aflibercept was more abundant than VEGF-bound aflibercept for the majority of the study.

With respect to the PK parameters of concomitant chemotherapy, irinotecan CL (CV%) was approximately 12 L/h/m^2

Table 5 Comparison of free and VEGF-bound aflibercept PK parameters at Cycle 1 between treatment responder and non-responders

	Free aflibercept PK parameters						VEGF-bound aflibercept PK parameters				
Population	N	C_{max} (μg/mL)	AUC (μg.day/mL)	CL (L/day)	CL/Weight (L/day/kg)	V_{SS} (L)	V_{SS}/Weight (L/kg)	N	C_{max} (μg/mL)	AUC_{last} (μg.day/mL)	$AUC_{0\text{-}2weeks}$ (μg.day/mL)
Responders (R)	6	58.6 (58.1) [14]	301 (296) [20]	0.907 (0.897) [16]	0.0137 (0.0135) [19]	6.31 (6.25) [15]	0.0950 (0.0940) [16]	6	2.63 (2.62) [7][b]	18.9 (18.9) [9][b]	17.3 (17.2) [11][c]
non-Responders (nR)	14	67.5 (65.4) [28]	286 (281) [19][a]	0.919 (0.881) [32][a]	0.0146 (0.0143) [23][a]	5.66 (5.35) [37][a]	0.0899 (0.0866) [29][a]	14	2.83 (2.75) [25]	21.3 (20.5) [31]	21.3 (20.2) [35][d]
R/nR Geometric Mean Ratio		0.89	1.06	1.02	0.95	1.17	1.09		0.95	0.92	0.85

Continuous variables are summarized as mean (geometric mean) [coefficient of variation %]. Patients presenting t_{last} outside ±15% of the 2-week dosing interval (i.e. 11.9–16.1 days) were excluded from calculation of descriptive statistics of VEGF-bound aflibercept C_{max} and AUC_{last} except for $AUC_{0\text{-}2weeks}$ when calculable (i.e. $t_{last} \geq 14$ days)

PK: pharmacokinetics

[a] n = 13

[b] n = 4

[c] n = 5

[d] n = 8

Table 6 Plasma trough concentration of free and VEGF-bound aflibercept and their ratio

Treatment Cycle	Cycle 2 (n = 17)	Cycle 3 (n = 12)	Cycle 5 (n = 8)	Cycle 7 (n = 7)	Cycle 9 (n = 3)	Cycle 11 (n = 2)
Free / VEGF-bound aflibercept ratio*	1.98 ± 0.499 (1.89) [25]	1.75 ± 0.769 (1.54) [44]	1.25 ± 0.646 (1.06) [52]	1.76 ± 0.506 (1.71) [29]	1.76 ± 1.37 (1.37) [78]	1.25 ± NC (0.988) [NC]

Continuous variables are summarized as mean ± SD (geometric mean) [coefficient of variation %]

NC: not calculated

*Any sample not drawn within the 2 week ±2 day time interval compared to start of infusion was not included in the calculation

(31%) and AUC was 15.2 μg.h/mL (20%). Its metabolite, SN-38, represents around 3% of the parent drug exposure on a molar basis. PK parameters for irinotecan and SN-38 for treatment responders and non-responders are presented in Supplementary Table 1. The 5-FU CL_{ss} (CV%) was around 79 L/h/m^2 (44%).

Discussion

This study represents the first assessment of the combination of aflibercept with FOLFIRI in Chinese patients with advanced, previously treated solid tumors. A dose of 4 mg/kg of aflibercept was selected based on the recommended dose determined by other studies within the international aflibercept clinical development program. [14–17, 20–22] The results of the present study indicate that 4 mg/kg of aflibercept with FOLFIRI is well-tolerated, has anti-tumor activity in pre-treated patients with solid tumors, especially in GI tumor types, and has a PK profile consistent with that observed in other clinical studies in non-Chinese patients.

The characteristics of patients in this study are comparable with those of previous Phase I studies of aflibercept with 5-FU, leucovorin and irinotecan (Table 1). In the two-part TCD6118 study of aflibercept in Caucasian patients with advanced solid tumors, the patients who received aflibercept 4 mg/kg had a median age of 54 and 55 years and most patients had a colorectal primary tumor site (70–75%) [14, 16, 22]. Although TCD6118 included only patients with an ECOG PS of 0, they were more heavily pre-treated, with a median of 3–4 lines of prior chemotherapy, compared with one line in this Chinese study.

Compared with the patients who received aflibercept 4 mg/kg in the Caucasian Phase I study (TCD6118), in Chinese patients the mean free aflibercept values for C_{max} (64.8 μg/mL) AUC (291 μg.day/mL), CL (0.92 L/day) and Vss (5.9 L) were similar, as indicated by Chinese to Caucasian geometric mean ratios close to 1 (Table 4). Although the PK parameters were numerically lower in Chinese patients, they showed lower variability compared with the Caucasian data. Conversely, the mean C_{max} and AUC_{last} values for VEGF-bound aflibercept were 1.4- and 1.3-fold higher, respectively, in Chinese patients compared with Caucasian patients, which may suggest that different amounts of endogenous VEGF are produced in these patient groups. Nonetheless, the mean free to VEGF-bound aflibercept concentration ratio was maintained at >1 over the majority of the study treatment duration in these previous patient populations and in the present study, indicating that the 4 mg/kg dose provides sufficient free aflibercept to be biologically active and block endogenous VEGF.

The addition of aflibercept to FOLFIRI did not influence the PK of the individual agents (irinotecan and 5-FU), confirming the findings of previous studies [14–17, 22]. In the additional exploratory analysis comparing PK parameters of aflibercept at Cycle 1 between treatment responders and non-responders, no noticeable differences were observed in the PK of free and VEGF-bound aflibercept, irinotecan or SN-38 between these two groups of patients (Table 5, Supplementary Table 1).

The most common TEAEs recorded in the present study were predominantly toxicities known to be associated with FOLFIRI chemotherapy, including decreased appetite, GI disorders (nausea, vomiting, diarrhea, abdominal pain and stomatitis), hematological abnormalities (leukopenia and neutropenia) and asthenia. Toxicities related to VEGF-inhibition, such as hypertension, hemorrhagic events, impaired wound healing, venous thrombosis and proteinuria were also reported. Grade 3 hypertension was reported in 6 patients (6/20, 30%), with no Grade 4 hypertension, which is in-line with findings of previous Phase I studies (Grade 3 hypertension, 30–58%) [14–17, 22]. All incidences of hypertension resolved after treatment with anti-hypertensive medications. Based on the observed AEs, the frequency and severity of the AEs of special interest, no deaths during the study period and a median treatment duration that was relatively long in a pre-treated patient population, the overall safety profile was consistent with that reported in previous studies. No new or unexpected AEs occurred in Chinese patients.

Preliminary anti-tumor effects were observed in six (30%) patients who achieved an objective response of PR in less than 2 months. These six patients included four with metastatic CRC, one with esophageal cancer (all with adenocarcinoma histopathology) and one EGJ patient with squamous carcinoma. The median PFS for the 15 CRC patients in this study was 5.95 months (95% CI: 5.29–8.77). Although the heterogeneity

of the patient population and the small patient number limits the conclusions that can be drawn on efficacy, the results from the present study are promising.

In conclusion, this study of Chinese patients with pretreated advanced solid malignancies shows that 4 mg/kg of aflibercept in combination with FOLFIRI is well-tolerated with preliminary anti-tumor activity and a PK profile consistent with results reported in early studies of Caucasian patients. In addition, no unexpected safety findings were observed. The combination of aflibercept and FOLFIRI in East-Asian patients has been further evaluated in the Phase III AFLAME study; a multi-national, randomized, double-blind, placebo-controlled study in second-line mCRC patients which has recently been completed with results pending publication.

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Compliance with ethical standards

Conflicts of interest Yingxin Li, Xing Sun, Samira Ziti-Ljajic, Dongmei Shi and Nathalie Le bail are employees of Sanofi. The other authors have no further conflicts of interest to declare.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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