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Phase I study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 with 5-fluorouracil and leucovorin: A safe combination

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ABSTRACT

We performed a phase I study with the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 combined with 5-fluorouracil and leucovorin (5-FU/LV) to determine safety profile and assess pharmacokinetic interactions. Patients with advanced solid malignancies received LV 20 mg/m^2 followed by 5-FU 425 mg/m^2 both administered intravenously in 15 min daily for 5 days every 4 weeks. ABT-510 was administered subcutaneously twice daily continuously from day 2 onwards. Blood and urine samples for pharmacokinetic analyses were collected at days 1, 5 and 22. Twelve patients received a total of 45 cycles of 5-FU/LV combined with ABT-510. ABT-510 dose levels studied were 50 and 100 mg. The combination was well tolerated, with a toxicity profile comparable to that of 5-FU/LV alone. At the dose levels studied no significant pharmacokinetic interactions were observed. These data indicate that ABT-510 administered twice daily subcutaneously can be safely combined with 5-FU/LV administered daily for 5 days, every 4 weeks.

1. Introduction

ABT-510 is a new angiogenesis inhibitor derived from the naturally occurring angiogenesis inhibitor thrombospondin-1. It is a parenterally available nonapeptide with potent in vitro and in vivo antitumour activity.[1,2] In two phase I studies exploring prolonged continuous administration of ABT-510 in patients with advanced solid malignancies, either in a once or twice daily subcutaneous administration schedule, ABT-510 was devoid of dose-limiting toxicities. Only mild toxicities mainly consisting of injection site reactions and fatigue were observed.[3,4] In one patient with a leiomyosarcoma partial remission and in a significant number of patients prolonged disease stabilization was observed. Plasma pharmacokinetics were linear across the dose ranges tested, without signs of drug accumulation following prolonged administration. Daily doses of twice daily 10 mg or above yielded plasma concentrations exceeding concentrations active in preclinical models and were maintained for several hours per day.[2–4] Currently, single agent phase II studies are being performed with ABT-510 in patients with renal cell carcinoma, soft tissue sarcoma and lymphoma.

Generally, angiogenesis inhibitors are expected to yield tumour growth inhibition rather than tumour shrinkage. Theoretically, the best antitumour yield is expected to occur in case of minimal tumour load, e.g. in the adjuvant setting

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following surgical and or radiotherapeutic treatment or in a situation of minimal residual disease following optimal cytoreductive treatment. In addition, combining cytotoxic agents with angiogenesis inhibitors has meanwhile proven to be an attractive approach.[5–7]

We chose to explore a combination of ABT-510 administered subcutaneously and 5-fluorouracil and leucovorin (5-FU/LV). The combination of 5-FU/LV has been used frequently for adjuvant treatment of node-positive colorectal cancer and treatment of advanced colorectal cancer. Toxicity is usually mild with stomatitis, diarrhoea and leukopenia being the most frequent reported adverse events.[8,9] In the present study we explored a combination of twice daily subcutaneous administration of ABT-510 continuously with short intravenous infusions of 5-FU/LV administered daily for 5 days every 4 weeks (Mayo Clinics Regimen[8]), to establish the safety profile of this combination and exclude clinically relevant pharmacokinetic interactions.

2. Patients and methods

2.1. Eligibility criteria

Patients with a histologically confirmed diagnosis of an advanced solid malignancy for whom standard therapy options did not exist or for whom the combination 5-FU/LV was considered an appropriate treatment were eligible. Additional eligibility criteria included: age $\geqslant$18 years; Eastern Cooperation Oncology Group (ECOG) performance status $\leqslant$2; an estimated life expectancy of $\geqslant$3 months; no radiotherapy, chemotherapy or hormonal therapy within 4 weeks before study start with the exception of small field radiation; and ability to receive subcutaneous injections of the study drug. Specific exclusion criteria included: a known human immunodeficiency virus positive status; a diagnosis of primary brain tumour or known central nervous system metastases; and evidence of uncontrolled clinically significant disease unrelated to the primary malignancy. The study was approved by local ethics boards of the two participating centres and all patients gave written informed consent.

2.2. Drug administration

The 5-FU/LV was administered intravenously as short infusions daily for 5 days, every 4 weeks. LV was administered over 15 min at a dose of 20 mg/m^2 dissolved in 100 mL 0.9% saline, followed by 5-FU over 15 min at a dose of 425 mg/m^2 dissolved in 100 mL 0.9% saline. ABT-510 (Abbott Laboratories, Chicago, IL, USA) administered twice daily subcutaneously was given from day 2 onwards, continuously. ABT-510 was supplied in vials containing 1.1 mL ABT-510 (100 mg/mL) or 0.75 mL ABT-510 (80 mg/mL) dissolved in dextrose 5%. The vials were stored at 2–8 °C and brought to room temperature 1 h before dosing. Patients injected themselves subcutaneously preferably at the same time in the morning and evening with an interval of approximately 12 h. The starting dose of ABT-510 was 50 mg twice daily, based upon safety and pharmacokinetic data obtained in single agent phase I studies with ABT-510.[3,4] No adjustments for body surface area or weight were made. Cohorts of six patients were studied.

Dose limiting toxicity (DLT) was defined as any grade 3 or 4 adverse event (except inadequately treated nausea or vomiting) or grade 2 adverse event requiring dose modification or treatment delay possibly or probably related to ABT-510 and occurring in the first treatment cycle (i.e. 4 weeks). Escalation of ABT-510 dose was pursued until either maximum tolerated dose (MTD) was identified or the highest recommended dose from the single agent study was reached (twice daily 100 mg). There was no dose escalation within an individual patient. The MTD was defined as the highest dose of ABT-510 given for at least one treatment cycle during which no more than one out of six patients would experience DLT.

2.3. Pre-treatment and follow-up studies

Prior to therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell count, including white blood cell differential, reticulocyte count, mean corpuscular volume, mean corpuscular haemoglobin, and serum biochemistry which involved sodium, potassium, chloride, magnesium, bicarbonate, creatinine, blood urea nitrogen, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, total bilirubin, calcium, phosphate, glucose, alkaline phosphatase and amylase were performed, as were prothrombin time, activated partial tromboplastin time, urinalysis, electrocardiogram and chest X-ray. Plasminogen, fibrinogen and factor VIII were collected at screening and thereafter only when clinically indicated. In addition, 24 h urine for albumin excretion was collected at screening and at day 22. Weekly evaluations during the first treatment cycle included history, physical examination, toxicity assessment according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0, complete blood count, prothrombin time, activated partial thromboplastin time and serum biochemistry. The same evaluations were also performed after 2 cycles and every 2 cycles thereafter. Additional visits were allowed at the discretion of the responsible physicians. Tumour measurements were performed every 2 cycles. Response was assessed using the World Health Organization (WHO) criteria for response.[10] Patients were allowed to continue treatment of both 5-FU/LV and ABT-510 in the absence of progressive disease or unacceptable toxicity. In case of discontinuation of 5-FU/LV due to toxicity, patients were allowed to continue treatment with ABT-510, provided there were no signs of disease progression.

2.4. Pharmacokinetic sampling and assays

For pharmacokinetic analysis of 5-FU 4.5 mL blood samples were collected in EDTA containing tubes from an indwelling intravenous canula contralateral to the site of the 5-FU infusion. Samples were collected prior to infusion of 5-FU, at the end of the 5-FU infusion and 5, 15, 30 min and 1, 2 and 4 h following the infusion of 5-FU on days 1 and 5 of cycle 1. Blood samples were immediately placed in an ice bath and plasma was separated by centrifugation. Plasma samples were stored in polypropylene tubes at −20 °C until analysis. The analysing method of 5-FU and its metabolite 5,6-dihydrofluorouracil

(FUH_2) has recently been described.[11] The lower limits of quantification in plasma for 5-FU and FUH_2 were 0.040 and 0.075 μg/mL, respectively. Blood samples (4.5 mL) for pharmacokinetic analysis of ABT-510 were collected in EDTA containing tubes prior to dosing of ABT-510 and 5, 15, 30 min and 1, 2, 4, and 8 h following the morning dose of ABT-510 on days 5 and 22 of cycle 1. On day 5 the morning dose of ABT-510 was administered about 30–60 min before the start of the infusion of LV. After collection, blood samples were placed on ice and plasma was separated by centrifugation after which plasma samples were stored in polypropylene tubes at −20 °C until analysis. Plasma concentrations of ABT-510 and its major metabolite M-1 were determined using a validated liquid chromatography with mass spectrometry (LC/MS/MS) method described previously.[3] The lower limits of quantification in plasma for ABT-510 and major metabolite M-1 were 0.5 and 3 ng/mL, respectively.

Urine for pharmacokinetic analysis of ABT-510 was collected on days 1, 5 and 22 of treatment cycle 1. Two samples of 15 mL were collected immediately prior to dosing of 5-FU/LV on day 1 for the base-line drug assay. Urine was sampled from 0 to 12 and 12 to 24 h after dosing of ABT-510 on days 5 and 22. The urine samples were refrigerated during the collection period and total volumes were measured. Two 15 mL samples from each sampling period were stored at −20 °C until analysis. For measurement of urine concentrations of ABT-510 and metabolite M-1, 200 μL of urine was processed according to previously described methods.[3] The lower limits of quantification in urine for ABT-510 and metabolite M-1 were 6 and 99 ng/mL, respectively.

2.5. Pharmacokinetic analysis

Non-compartmental methods were used to determine values of pharmacokinetic variables of 5-FU, FUH_2, ABT-510 and metabolite M-1 using WinNonlin-Pro™, version 4.1 (Pharsight Corporation, Cary, NC, USA). The maximum observed plasma concentration and time of maximum observed plasma concentration were reported as C_{max} and T_{max}, respectively. The value of the terminal elimination rate constant (β) was obtained from the slope of the linear regression of the logarithms of the plasma concentration versus time data from the terminal log-linear phase of the profile. The terminal log-linear phase was identified using WinNonlin-Pro and visual inspection. The terminal phase elimination half-life ($t_{1/2}$) was calculated as ($\ln 2/\beta$). The area under the plasma concentration–time curve over an ABT-510 dosing interval ($AUC_{0-12\ h}$) was calculated by the linear trapezoidal rule, while the concentration at 12 h was set equal to the predose concentration. The AUC from zero to infinite time ($AUC_{0-\infty}$) was calculated by adding AUC from time zero to the time of the last measurable concentration to AUC_{ext}, where AUC_{ext} was calculated by dividing the last measurable concentration by β. Clearance (CL) or apparent clearance (CL/F) was calculated by dividing the dose by the AUC, and the volume of distribution (V_Z) or apparent volume of distribution (V_Z/F) was calculated by dividing CL or CL/F by β. The fraction of the dose recovered in urine as ABT-510 and M-1 was calculated as the amount recovered in urine over the dosing interval divided by the dose. The amount of M-1 recovered in urine was converted to equivalent ABT-510 amount by multiplying by the ratio of molecular weights (994/501).

2.6. Statistics

The sample size was based on clinical justification and patient numbers historically used for testing of new anti-neoplastic compounds. In models for the analysis of safety data, dose was treated as a factor with discrete levels or as a continuous variable. For the pharmacokinetic analysis, descriptive statistics of parameters were determined with a breakdown by regimen and dose level on days 1, 5 and 22.

3. Results

Twelve patients, whose characteristics are listed in Table 1, received a total of 45 cycles (median 4, range 1–7) of 5-FU/LV in combination with ABT-510. All patients were evaluable. ABT-510 dose levels studied were twice daily 50 mg (6 patients) and twice daily 100 mg (6 patients).

Table 1 – Patient characteristics

No. of patients included	12
Male/female	9/3
Median age	54
Range, years	47–78
WHO performance status	
0	5
1	7
Prior chemo/immuno/hormonal therapy	
0–3 prior regimens	10
⩾4 prior regimens	2
Prior radiotherapy	7
Tumour type	
Colorectal carcinoma	2
Head and neck cancer	2
Renal cell carcinoma	2
Non-small cell lung cancer	1
Oesophageal carcinoma	1
Carcinoma unknown primary	1
Colorectal and ampulla of Vater carcinoma	1
Synovial sarcoma	1
Thymus carcinoma	1

Table 2 – Grades 3 and 4 adverse events observed during the study, no. of patients (%)

	ABT-510 dose (twice daily)		
	50 mg (n = 6)	100 mg (n = 6)	All doses (n = 12)
Diarrhoea	1 (17%)	1 (17%)	2 (17%)
Fatigue	0	1 (17%)	1 (8%)
Atrial fibrillation	1 (17%)	0	1 (8%)
Dyspnoea	0	1 (17%)	1 (8%)
Bilirubinemia	1 (17)	0	1 (8%)
Transaminase	1 (17%)	1 (17%)	2 (17%)
Hyperglycaemia	0	1 (17%)	1 (8%)
Neutropenia	4 (67%)	4 (67%)	8 (67%)

3.1. Toxicity

The frequency of grades 3 and 4 adverse events is listed in Table 2. The frequency of grades 3 and 4 adverse event is considered as normal for administration of 5-FU/LV at this dose in a daily five-day schedule. None of these adverse events were considered to be possibly or probably related to ABT-510, with the exception of one episode of atrial fibrillation in a patient with rectal carcinoma. The patient was hospitalised on day 11 of the first cycle with febrile neutropenia and grade 2 mucositis and treated with broad spectrum antibiotics. The atrial fibrillation occurred on day 14 and was successfully treated with medication. Grades 1 or 2 adverse events possibly or probably related to ABT-510 and occurring in more than one patient were fatigue (25%), injection site reactions (33%) and dizziness (17%).

3.2. Pharmacokinetics

Plasma samples for pharmacokinetic analysis of 5-FU (days 1 and 5) and ABT-510 (days 5 and 22) were available from all patients, and results are summarized in Tables 3 and 4. The pharmacokinetics of 5-FU were largely unaffected by co-administration with ABT-510, including no significant effect on 5-FU C_{max} or AUC (Table 3 and Fig. 1). Similarly, ABT-510 pharmacokinetics were not affected by co-administration

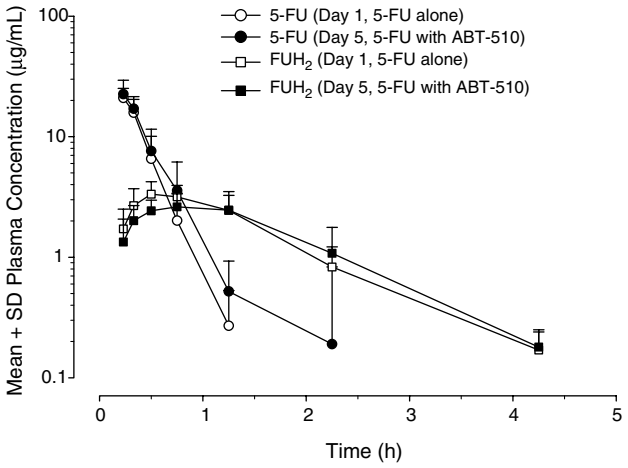

Fig. 1 – Mean (SD; n = 12) plasma concentration–time profiles of 5-FU and metabolite FUH_2 without (day 1) and with (day 5) co-administration of ABT-510 (either ABT-510 dose levels).

Table 3 – Mean (SD) pharmacokinetic parameters of 5-FU and FUH_2 without (day 1) and with (day 5) co-administration of ABT-510 following administration of 5-FU over 15 min at a dose of 425 mg/m²

	5-FU		FUH_2	
	Day 1 (n = 12)	Day 5 (n = 12)	Day 1 (n = 12)	Day 5 (n = 12)
T_{max} (h)[a]	0.25 (0.04)	0.28 (0.05)*	0.61 (0.15)	0.86 (0.52)
C_{max} (µg/mL)	22.2 (3.5)	22.2 (6.6)	3.7 (0.9)	2.9 (0.6)**
$AUC_{0-\infty}$ (µg h/mL)	7.9 (1.6)	9.2 (2.7)	6.0 (1.8)	5.6 (1.6)
$t_{1/2}$ (h) [b]	0.1 (0.0)	0.2 (0.1)**	0.8 (0.1)	0.8 (0.1)
CL (L/h/m²)	56.2 (11.9)	50.2 (16.0)	NC	NC
V_Z (L/m²)	11.6 (2.7)	14.3 (5.7)	NC	NC

a Abbreviations: T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; $AUC_{0-\infty}$, area under the plasma concentration versus time curve from time zero to infinite time; $t_{1/2}$, half-life; CL, clearance; V_Z, volume of distribution; NC, not calculated.
b Harmonic mean ± pseudo standard deviation.
* $P < 0.05$.
** $P < 0.01$.

Table 4 – Mean (SD) pharmacokinetic parameters of ABT-510 with (day 5) and without (day 22) co-administration of 5-FU/LV

	ABT-510 dose (twice daily)			
	50 mg		100 mg	
	Day 5 (n = 6)	Day 22 (n = 6)	Day 5 (n = 6)	Day 22 (n = 6)
T_{max} (h)[a]	0.67 (0.26)	0.67 (0.38)	0.50 (0.27)	0.75 (0.27)
C_{max} (ng/mL)	997 (493)	1181 (366)	1877 (513)	1789 (708)
AUC_{0-12} (ng h/mL)	2204 (885)	2445 (705)	5137 (1332)	5338 (2077)
$t_{1/2}$ (h)[b]	1.0 (0.1)	1.0 (0.2)	1.1 (0.3)	1.3 (0.4)
CL/F (L/h)	25.8 (10.3)	21.9 (6.3)	20.8 (6.4)	20.8 (6.7)
V_Z/F (L)	36.1 (12.0)	33.1 (6.0)	33.5 (12.0)	42.8 (22.0)

a Abbreviations: T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; AUC_{0-12}, area under the plasma concentration versus time curve over a dosing interval (from time 0 to 12 h); $t_{1/2}$, half-life; CL/F, apparent clearance; V_Z/F, apparent volume of distribution.
b Harmonic mean ± pseudo standard deviation.

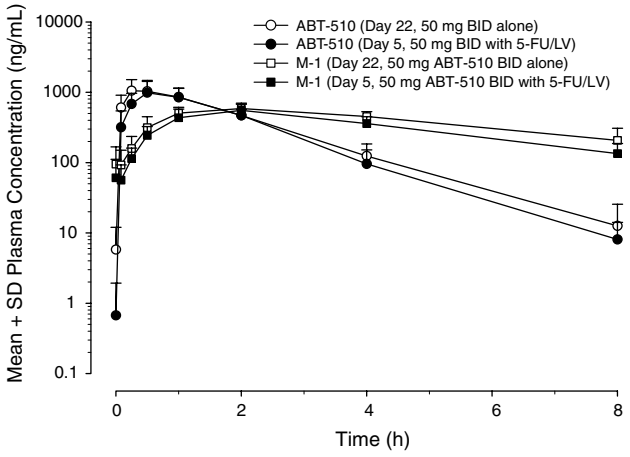

Fig. 2 – Mean (SD; *n* = 6) plasma concentration–time profiles of ABT-510 and metabolite M-1 with (day 5) and without (day 22) co-administration of 5-FU/LV following the morning dose of ABT-510 50 mg twice daily (BID).

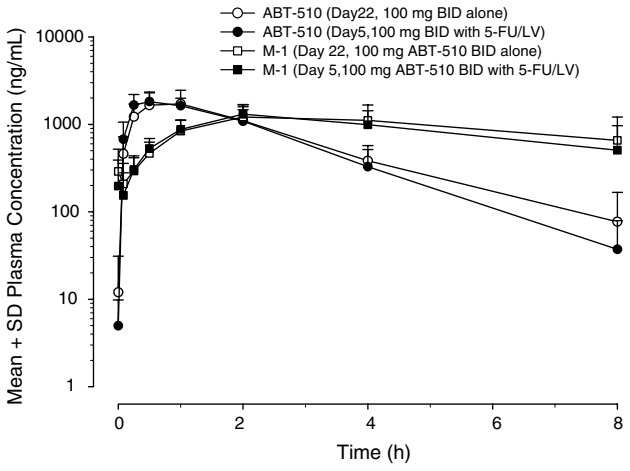

Fig. 3 – Mean (SD; *n* = 6) plasma concentration–time profiles of ABT-510 and metabolite M-1 with (day 5) and without (day 22) co-administration of 5-FU/LV following the morning dose of ABT-510 100 mg twice daily (BID).

with 5-FU (Table 4 and Figs. 2, 3), including the amount of ABT-510 and M-1 recovered in urine (data not shown). Together, these results indicate no pharmacokinetic interaction when ABT-510 is co-administered with 5-FU at the studied doses.

3.3. *Antitumour activity*

No tumour regressions were observed. Stable disease for more than 4 cycles was found in four patients (33%) diagnosed with non-small cell lung cancer, colorectal cancer, renal cancer and head and neck cancer. One patient with colorectal cancer withdrew consent after cycle 7 (28 weeks) and was shown to have stable disease at 30 weeks.

4. Discussion

In this phase I study we explored the feasibility of combining the new angiogenesis inhibitor ABT-510 with 5-FU/LV administered as a short intravenous infusion daily for 5 days every 4 weeks. It was shown that ABT-510 administered at doses of 50 and 100 mg twice daily subcutaneously (the recommended single agent doses) could be safely combined with this 5-FU/LV regimen. The observed toxicity in this study was comparable to that following treatment with 5-FU/LV alone.[8,9] Given the small number of patients in this study, we can obviously not exclude the possibility of rare side effects occurring due to this combination. Therefore, further studies on the combination should still include close monitoring of toxicity. In addition to the clinical safety profile, there were no relevant pharmacokinetic interactions. Although it cannot be excluded that differences in T_{max} and $t_{1/2}$ of 5-FU on day 5 compared to day 1 could have been due to an interaction with ABT-510, this observed differences are only very small, whereas overall drug exposures was unchanged. The lack of pharmacokinetic interaction observed in this study is consistent with the different disposition pathways involved in the elimination of ABT-510 and 5FU/LV.

While we believe that 5-FU/LV can be safely combined with the recommended single dose of ABT-510 given twice daily subcutaneously, a discussion could evolve as to which dose of ABT-510 to use in further clinical efficacy studies. Defining the optimal dose for angiogenesis inhibiting agents is challenging since the maximum tolerated dose frequently can not be assessed, due to a lack of severe toxicity. Surrogate endpoints such as circulating levels of pro-angiogenic factors (e.g. vascular endothelial growth factor [VEGF] and basic fibroblast growth factor [bFGF]) and/or imaging assessments on changes in tumour blood flow could be helpful in determining the optimal biological dose. Unfortunately, neither of these methods has been validated so far. In our phase I study with single agent ABT-510 we observed a significant decrease of bFGF but no relevant changes in VEGF and IL-8.[3] However, the changes of bFGF observed were independent of the ABT-510 dose and did not correlate with duration of treatment. For several other naturally occurring angiogenesis inhibitors, animal tumour models suggested that antitumour and angiogenesis inhibiting activity may improve with continuous infusion compared with bolus administration.[12,13] For ABT-510, the preclinical models suggest that the efficacy following continuous subcutaneous infusions is similar compared to subcutaneous bolus doses. In addition, studies in 11 different murine antitumour models showed that 75% of maximal activity was reached when plasma concentrations exceeded 100 ng/mL for at least 3 h a day.[2] Therefore, time over this 100 ng/mL threshold per day is the major element for selecting the optimal dose of ABT-510. This exposure could be reached with a dose of 10 mg twice daily or higher.[3,4]

It is important to explore the various possible ways of using angiogenesis inhibitors. One way might be to use them in the adjuvant setting, after primary curative surgical resection. However, this will require long lasting large studies as well as more convincing data on activity and more information on long term safety of ABT-510 than is currently available. In view of the currently obtained data it

is also conceivable to explore its use in metastatic or advanced cancer to yield long lasting absence of progression. Yet another way would be using angiogenesis inhibitors after primary cytoreductive chemotherapy for advanced disease, to prolong the time to progression. Finally it is potentially worthwhile to combine angiogenesis inhibitors with cytotoxic treatment, the first evidence of which has lead to registration of bevacizumab, a recombinant humanized monoclonal antibody to VEGF, for treatment of metastatic colorectal cancer in combination with a 5-FU containing regimen.[5,6] Combining 5-FU/LV with ABT-510 is attractive because of different mechanisms of action, lack of overlapping toxicity and the practical feasibility of this combination. However, the use of such combinations would require special attention since recent experiences with several other angiogenesis inhibiting agents tested in combination with 5-FU/LV revealed unexpected major toxicities. For example, SU5416, a VEGF receptor tyrosine kinase inhibitor, which was studied in a phase I/II setting combined with 5 FU/LV.[14] While this study did not reveal any dose-limiting toxicity, a subsequent randomised phase III study of 5-FU/LV with or without SU5416 in patients with metastatic colorectal cancer had to be terminated prematurely due to an unexpected high incidence of thromboembolic events in the SU5416 arm. A similar, albeit less pronounced observation was reported for bevacizumab combined with 5-FU/LV.[15] Similar problems have not been encountered in our current small study. Importantly the rate of thromboembolic events with ABT-510 is minimal,[3,4] contrasting the above experience with other angiogenesis inhibitors. Whether this relates to differences in mechanism of action is currently unknown.

In conclusion, we found that ABT-510 administered twice daily subcutaneously continuously could be combined safely with 5-FU/LV administered daily intravenously for 5 days every 4 weeks. Clinically relevant pharmacokinetic interactions were not observed. In view of the lack of additional toxicity, the ease of administration and the interesting clinical activity observed in single agent studies with ABT-510, additional efficacy studies with this combination seem warranted.

Conflict of interest statement

R. Knight, R.A. Carr and R. Humerickhouse are employees of Abbott Laboratories, Chicago, IL, USA.

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