Phase I, pharmacokinetic, and bone marrow drug-level studies of trimonthly 48-h infusion of high-dose 5-fluorouracil and leucovorin in patients with metastatic colorectal cancers

Yunn-Fang Ho[a,b], Wan-Chen Lu[a], Russel Rhei-Long Chen[b], Ann-Lii Cheng[c,d,e] and Kun-Huei Yeh[c,d]

The primary aim of the high-dose 5-fluorouracil (5-FU) and leucovorin (LV; HDFL48) phase I study was to determine the maximum tolerated dose and dose-limiting toxicity of 5-FU and LV with modified trimonthly 48-h continuous infusion of high-dose 5-FU/LV in patients with metastatic colorectal cancer. The study also determined the pharmacokinetic parameters of 5-FU, especially steady-state plasma and bone marrow (BM) concentrations. Eligibility included serum triglyceride of more than or equal to 70 mg/dl, adequate BM function, and the major typical trial criteria. Sixteen patients who were enrolled received trimonthly 5-FU at 2500 mg/m^2/48 h/week (with 500 then 250 mg/m^2/48 h/week escalation by a conventional 3–3 schema up to 3750 mg/m^2/48 h/week) and LV at 300 mg/m^2/48 h on days 1, 8, and 15 during a regular 28-day cycle. The maximum tolerated dose of 5-FU was 3750 mg/m^2/48 h/week with this trimonthly schedule. Dose-limiting toxicities were grade III neutropenia with more than 1-week delay of the next cycle, grade III mucositis, diarrhea, and hand–foot syndrome for more than 3 days. The regimen maintains significant concentration differences between steady-state plasma and BM concentrations (8.06 ± 6.39 vs. 2.89 ± 1.01 μmol/l, $P = 0.021$) as measured by high-performance liquid chromatography. Toxicities were of minor grade and were tolerable, with minimal myelotoxicity significantly associated with low steady-state BM concentration. None had 5-FU-related hyperammonemic encephalopathy. Three patients had an objective partial response (18.8%, 95% confidence interval: 4–46%) and two of the 14 patients, who had failed HDFL24 (2600 mg/m^2/24 h/week), had a partial response to HDFL48 (14.3% partial response, 95% confidence interval 2–43%). Median progression-free survival and overall survival were 4.1 months (range: 1.8–12.5) and 10.5 months (range: 2.7–32.1), respectively. The efficacy and low myelotoxicity of HDFL48 were attributed to the sustained adequate steady-state plasma concentration and an average 2.63-fold concentration gradient between plasma and BM compartments at steady state. The recommended 5-FU dose for use in future trials was 3500 mg/m^2/48 h/week, with a fixed dose of LV at 300 mg/m^2/48 h/week. *Anti-Cancer Drugs* 22:290–298 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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[a]Graduate Institute of Clinical Pharmacy, [b]School of Pharmacy, [c]Graduate Institute of Oncology, National Taiwan University College of Medicine, [d]Department of Oncology and [e]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, Republic of China

Correspondence to Kun-Huei Yeh, MD, PhD, Department of Oncology, National Taiwan University Hospital, Graduate Institute of Oncology, National Taiwan University College of Medicine, No. 7, Chung-Shan S. Road, Taipei 100, Taiwan, Republic of China
Tel: +886 2 23123456 x67514; fax: +886 2 23711174;
e-mail: khyeh@ntu.edu.tw

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Introduction

Colorectal cancer (CRC) ranks as the second most common malignancy in terms of incidence and mortality in developed countries [1], with approximately 1 million new diagnoses and half a million deaths worldwide annually. The 5-year survival for CRC is approximately 50–60% after appropriate treatment [1]. Continued development of effective chemotherapy regimens is essential for a reduction of disease recurrence and prolongation of patient survival.

The fluorinated pyrimidine 5-fluorouracil (5-FU), an antimetabolite synthesized in 1957 [2], has long been used in chemotherapy for CRCs [3]. The cytotoxicity of 5-FU seems to be associated primarily with its converted metabolite 5-fluorodeoxyuridine monophosphate, which binds tightly to thymidylate synthase in the presence of a reduced folate cofactor and, thereby prevents DNA synthesis [4]. Leucovorin (LV, folinic acid, 5-formyl tetrahydrofolate), by ensuring the thymidylate synthase–5-fluorodeoxyuridine monophosphate–folate complex formation, is commonly supplemented as a biomodulator in 5-FU regimens to enhance its efficacy. Recent strategies combining 5-FU/LV with newer antineoplastic agents, such as irinotecan and oxaliplatin, have shown superior response rates (RRs) when compared with 5-FU/LV alone [5–7]. Nevertheless, the 5-FU-based infusion regimen remains the cornerstone for the treatment of advanced CRC and has been advocated by oncologists worldwide [4,8].

5-FU is renowned for its diverse intrapatient and interpatient variability in plasma levels and therapeutic responses [9]. Numerous attempts have been made to improve

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the efficacy and toxicity profiles of 5-FU through modifications in dose intensity [10–12], route of administration [13], administration schedule [3,10,13], biomodulator selection [14,15], and special pharmacokinetics-guided dosage adjustment [16,17]. Augmented by both in-vitro and clinical evidence [18,19], it is generally accepted that a longer and sustained exposure to 5-FU plasma concentration through continuous infusion together with LV results in superior tumor response and less toxicity.

The maximum tolerated doses (MTDs) for 5-FU in protracted 28-day continuous infusion and in weekly high-dose 5-FU/LV 24-h continuous infusion protocols (HDFL24) were determined to be 450 mg/m^2/day [20] and 2600 mg/m^2/24 h/week [21], respectively. The latter HDFL24 regimen, sometimes with LV dosage reduction, has been adopted by oncologists, resulting in satisfactory responses without calcite precipitation [22,23]. Furthermore, clinical research groups in Canada [10], Spain [15,24–26], and France [12,14,27] have evaluated a 48-h continuous infusion 5-FU schedule. However, this regimen resulted in variable outcomes depending on the 5-FU dosage (2000–4000 mg/m^2/48 h), administration frequency (weekly vs. bimonthly), presence of LV modulation (with vs. without), LV dosage (0–500 mg/m^2/day), and route of LV administration (oral vs. intravenous).

The optimal 5-FU infusion dose and schedule remain unclear. Intriguingly, data from an in-vitro study suggest that a 5-FU concentration of 2.5 μmol/l, a level less than half of that achieved by 24-h infusion of 5-FU at 2600 mg/m^2, still effectively suppressed free thymidylate synthase ([18] data not shown). Our preliminary results also indicate that exposure of cancer cells to 2.5 μmol/l of 5-FU for more than 24 h may result in even higher cytotoxicity. At this serum concentration, we do not expect any increment of myelotoxicity [19], with the major concerns being hand–foot syndrome and mucositis. Clinical evidence indicates that weekly schedules significantly alleviate the severity of mucositis. Furthermore, a resting period of 1 week between each cycle helps relieve hand–foot syndrome [23]. Taken together, we hypothesize that HDFL48, by sustaining effective 5-FU concentration in plasma, yet sequestering bone marrow (BM) from 5-FU infiltration, would further improve the efficacy of HDFL24 regimens without causing significantly more toxicity. The results of the bimonthly 48-h infusion protocol used by de Gramont *et al.* [12,14,27] seem to support this suggestion. These findings may form the basis for further exploration of the optimal dose/schedule for the treatment of CRC with 5-FU infusion. To optimize an antineoplastic therapy for metastatic CRC, a trimonthly 48-h infusion of high-dose 5-FU and LV (HDFL48) was designed with the goal of achieving a longer, sustained exposure of tumors to a cytotoxic concentration of 5-FU.

The primary objective of this phase I pharmacokinetic (PK) and BM study was to determine the MTD and dose-limiting toxicity (DLT) of 5-FU and LV with this novel trimonthly 48-h infusion schedule, and to collect patient toxicity profiles at different dose levels of 5-FU/LV 48-h infusion. A secondary objective was to study the PK parameters of 5-FU, in particular steady-state plasma (C_{SS}) and BM (C_{BM}) concentrations, during this 48-h continuous infusion regimen with 5-FU dosage escalations and fixed-dose LV. Finally, patient data were used to show that an absence of myelosuppression under this regimen of prolonged infusion may be related to the resultant lower BM levels of 5-FU.

Patients and methods

Study design and patient eligibility

This phase I clinical study was approved by the Research Ethics Committee of the National Taiwan University Hospital, Taipei, Taiwan. A typical cycle of trimonthly HDFL48 was initiated with 5-FU (2500 mg/m^2/48 h/week; with dosage escalation) and LV (300 mg/m^2/48 h/week fixed dose) in normal saline by intravenous infusion (Table 1). After three doses of HDFL48 (days 1, 8, 15), patients rested for 1 week, and then the subsequent cycle was started on day 29. The dose of 5-FU was determined at the entry level. A conventional 3–3 dose-escalation schema was used to determine the MTD of this combination; namely, cohorts of three patients were treated at each dose level. If no DLT was seen at a given dose level, the dose was escalated for the next cohort. If the incidence of DLT was 33%, then three more patients were treated at the same level. If no further patients of DLT were seen in the additional patients, then the dose level was escalated for the next cohort. Otherwise, dose escalation stopped at two of six patients with DLT. If the

Table 1 **Dosage escalation schema**

	Study protocol (mg/m^2/48 h/week)		Actual dosage administered (mg/48 h/week; $n=17$[a])		
Dosage levels	5-fluorouracil	Leucovorin	5-fluorouracil	Leucovorin	(*n*)
I	2500	300	4066.7 ± 635.6	483.3 ± 76.4	(3)
II	3000	300	4719.0 ± 715.1	470.8 ± 71.2	(4)
III	3500	300	5737.5 ± 384.0	489.5 ± 32.9	(4)
IV	3750	300	6151.7 ± 744.7	499.8 ± 47.2	(6)

Data are expressed as mean ± standard deviation.
[a]One patient had participated trials of both I and II dosage levels.

incidence of DLT was greater than 33% at a given level, then dose escalation also stopped. MTD was defined as the dose level at which two or more of the six patients experienced DLT. The recommended phase II dose was defined as one dose level below the MTD. Patients were de-escalated in the next cycle if DLT had been reached and continuous treatment was indicated. Intrapatient dose escalation was allowed in subsequent cycles for patients with stable disease after completion of two cycles, as a higher dose of 5-FU is possibly associated with better efficacy for patients with stable disease.

Patients who signed consent were enrolled from the outpatient clinics and the inpatient wards of the Department of Oncology, National Taiwan University Hospital. All patients were 18 years of age or older. The inclusion criteria were pathologically proven metastatic colorectal adenocarcinoma indicated for 5-FU chemotherapy or failure before 5-FU therapy of other schedules, at least one measurable tumor by image studies, Karnofsky performance status of more than or equal to 50%, fasting serum triglyceride of more than or equal to 70 mg/dl [28], adequate BM function (leukocytes $\geq$ 3000/μl or absolute neutrophil counts $\geq$ 1500/μl, and platelets $\geq$ 75 000/μl), renal function (serum creatinine $\leq$ 1.5 mg/dl and proteinuria $\leq 1+$), hepatic function (serum total bilirubin and transaminase $\leq 3.5\times$ the upper limits of reference values), and at least a 4-week interval after earlier chemotherapy or radiotherapy. A fasting serum triglyceride level of less than 70 mg/dl was considered to be an important risk factor for HDFL-related neurotoxicity [28]. Fasting triglyceride data were checked by a central laboratory during an interval of not more than 7 days before the start of HDFL48. Major exclusion criteria included concomitant antineoplastic therapy or radiotherapy, central nervous system metastasis, unacceptable mental status, pregnancy, and major systemic diseases.

Evaluation of toxicity and efficacy

The safety and efficacy profiles of the trimonthly HDFL48 therapy were evaluated during the study. The assessments before chemotherapy included obtaining a detailed medical history, physical examination, complete blood counts, blood chemistry tests, chest radiograph, and computed tomographic scans of the abdomen and pelvis. After the initiation of protocol treatment, complete blood counts were evaluated weekly and blood chemistry was assessed every 2 weeks. Patient condition and treatment-related toxicities were evaluated weekly. Tumor size was measured every 8 weeks by imaging, or when there were any clinical signs of possible tumor progression. Tumor response was evaluated according to the World Health Organization criteria. Toxicities were graded by using the National Cancer Institute-Common Toxicity Criteria (National Cancer Institute-CTC, version 2.0).

DLT was defined as the occurrence of any one of the following: (i) hematological toxicities, including (a) grade IV neutropenia (absolute neutrophil count $<$ 500/μl) or thrombocytopenia (platelet $<$ 25 000/μl) for more than 3 days, (b) febrile neutropenia (absolute neutrophil count $<$ 500/μl with body temperature $>$ 38.5°C) or neutropenic sepsis, or (c) neutrophil count fails to reach 1500/μl or platelet count fails to reach 75 000/μl by day 36 (i.e. more than 1-week delay of the scheduled next cycle of trimonthly HDFL48); or (ii) nonhematological toxicities including (a) any grade IV toxicity, (b) grade III neurological toxicity for more than 3 days, (c) any grade III nonhematological, non-neurological toxicity for more than 3 days, or (d) any grade III/IV toxicity by day 36 (i.e. more than 1-week delay of the scheduled next cycle of trimonthly HDFL48).

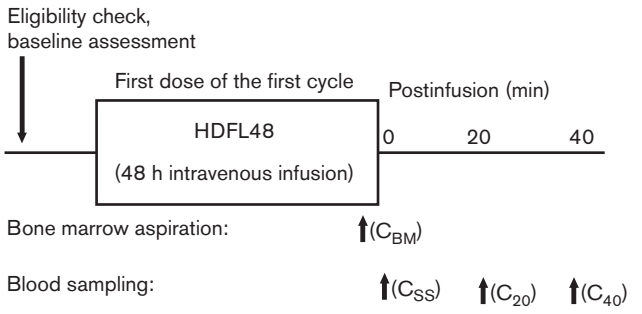
Materials

5-fluorouracil (5-FU, fluoro-uracil) and leucovorin (LV, rescuvolin) for human use were manufactured by Hoffmann-La Roche (Nutley, New Jersey, USA) and PCH Pharmachemie (Swensweg, Haarlem, The Netherlands), respectively. Reagents for high-performance liquid chromatography (HPLC) analysis, including 5-FU, 5-bromouracil, uracil, and diethyl ether (HPLC grade), were from Sigma (St Louis, Missouri, USA). Potassium dihydrogen phosphate, 85% orthophosphoric acid, and other HPLC-grade reagents, including acetonitrile, methanol, *n*-propanol, and isopropanol were provided by Merck (Darmstadt, Germany). Highly purified water produced by a Millipore Direct-Q5 system (Billerica, Massachusetts, USA) was used for all preparations.

Pharmacokinetic study and sample preparation

Blood samples (5 ml/each) were collected through indwelling intravenous cannulae in heparinized tubes at the end of the first dose of the first cycle of HDFL48 (C_{SS}), and 20 (C_{20}) and 40 min (C_{40}) postinfusion to assess the pharmacokinetic characteristics of 5-FU (Fig. 1). Using the standard BM aspiration technique from iliac crests or the sternum, we completed the BM

Fig. 1



Pharmacokinetic study design. C_{BM}, steady-state bone marrow concentration; C_{SS}, steady-state plasma concentration; HDFL, high-dose 5-fluorouracil and leucovorin.

aspiration procedure immediately before the completion of the first dose of the first cycle of HDFL48. BM aspirate (5 ml/each) was collected in heparinized tubes. Both blood and BM samples were immediately centrifuged at 3000 *g* for 5 min. The resulting supernatants (plasma) were stored at −80°C until assayed. All plasma samples were further processed for HPLC analysis according to the method of Escoriaza *et al.* [29]. Moreover, a final purification step involving centrifugal filtration with 0.45 μm low-binding Durapore membrane (Ultrafree-MC, Millipore, USA) was carried out immediately before sample injection into the HPLC system.

Liquid chromatography

Plasma samples were assayed for 5-FU using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, California, USA) equipped with a quaternary pump (G1311A, with continuous seal wash), diode array detectors (G1315B, multiple wavelength), a thermostatted autosampler (G1330A), a thermostatted column compartment (G1316A), and a vacuum degasser (G1322A). Instrument control and data evaluation were carried out using the Agilent ChemStation (Agilent Technologies) for the LC System. 5-FU concentration was determined as described earlier [29]. Chromatographic separation was carried out on a LiChrospher RP-18 analytical column (250 mm × 4.6 mm, 5 μm particle size; Supelco, Philadelphia, Pennsylvania, USA), with a small guard column containing the same material (4.0 × 3.0 mm). The mobile phase was 50 mmol/l of KH_2PO_4 adjusted to pH 3.0 with 85% orthophosphoric acid. The total run time of the analysis was 30 min. The flow rates and respective temperatures were as follows: 0–10 min, 1 ml/min at 25°C; 10–20 min, 1.7 ml/min at 17°C; and 20–30 min, 1 ml/min at 25°C. Sample detection was done at 266 nm with an injection volume of 20 μl.

Pharmacokinetic analysis

Pharmacokinetic parameters of 5-FU were determined based on linear pharmacokinetics with first-order elimination. The parameters assessed included 5-FU-C_{SS}, concentration at the end of infusion; 5-FU-C_{BM}, also concentration at the end of infusion; terminal-phase rate constant (k), slope of semilog plot of C_{SS}, C_{20}, C_{40} versus time; terminal half-life ($t_{1/2} = 0.693/k$); plasma clearance (Cl = infusion rate/C_{SS}); and apparent volume of distribution (V_d = Cl/k).

Statistical analysis

Patients who were evaluable for response were those who had completed at least two cycles of treatment. All enrolled intent-to-treat patients were subjected to toxicity evaluations. Progression-free survival (PFS) was defined as the duration from the date of protocol treatment initiation to the date of documented disease progression or death by any cause. Overall survival was defined as the duration from the date of protocol treatment initiation to the date of death. The Kaplan–Meier method was used in all survival analyses.

The significance of differences in PK parameters among the different compartments or subsets of patients was estimated by t-test, with P values of less than 0.05 considered to be statistically significant. All values were expressed as mean ± standard deviation. Statistical analyses were done using SAS software (v.8.01; SAS Institute, Cary, North Carolina, USA) for regression analysis.

Results

This study used four different 5-FU dosage levels, ranging from 2500 to 3750 mg/m^2/48 h/week. Sixteen patients (10 men and six women) at 17 person-dosage-levels were enrolled in the study. The mean patient age was 51.5 years (range: 27–80 years). In one patient, whose C_{SS} was extraordinarily low, and had an intrapatient dosage escalation from level I to II, and the higher dosage of 5-FU was possibly associated with a better chance of response. The actual doses of 5-FU that were administered were 4066.7 ± 635.6, 4719.0 ± 715.1, 5737.5 ± 384.0, and 6151.7 ± 744.7 mg/48 h/week for dosage levels I, II, III, and IV, respectively (Table 1).

Patient demographics and disease status are summarized in Table 2. All of these patients with CRC had metastases, with the liver being the most frequent metastatic site. Patients had undergone various treatments earlier, including surgery (87.5%), chemotherapy (93.8%), and radiotherapy (37.5%). Earlier chemotherapeutic regimens received included HDFL24, tegafur–uracil, irinotecan, irinotecan plus 5-FU/LV, irinotecan plus HDFL24, or oxaliplatin plus HDFL24. Only one patient was chemonaive. Eight of the 16 patients (50%) had failed oxaliplatin and/or irinotecan-based regimens (six patients: both oxaliplatin and irinotecan; seven: oxaliplatin; and seven: irinotecan), and they had benefits in terms of median PFS of 3.8 months (range 2.0–11.0) under HDFL48 treatment.

The MTD of 5-FU, the primary objective of this study, was established at 3750 mg/m^2/48 h/week, with a fixed dose of LV at 300 mg/m^2/48 h/week, for this novel trimonthly 48-h infusion HDFL48 schedule. DLT for this 5-FU/LV schedule occurred in two out of six patients receiving treatment level IV. One patient developed grade III neutropenia (absolute neutrophil count < 500/μl) and the patient's neutrophil counts failed to reach 1500/μl by day 36 (i.e. more than 1-week delay of the scheduled next cycle of trimonthly HDFL48). Another patient developed grade III mucositis, diarrhea, and hand–foot syndrome for more than 3 days. None of the 16 patients developed grade IV neutropenia, neutropenic sepsis, or 5-FU-related hyperammonemic encephalopathy in a total of 80 trimonthly cycles administered. For a future phase II study, the

Table 2 Patient characteristics

	HDFL48 dosage levels			
	I ($n=3$)	II ($n=4$[a])	III ($n=4$)	IV ($n=6$)
Age (mean)	55.3 (44 – 72)	49.8 (27 – 75)	55.8 (39 – 80)	54.8 (41 – 70)
Male, *n* (%)	2 (67)	3 (75)	3 (75)	3 (50)
BSA (m^2)	1.63 (1.35 – 1.85)	1.57 (1.22 – 1.75)	1.65 (1.50 – 1.76)	1.65 (1.34 – 1.84)
KPS (%)	83.3 (80 – 90)	85 (70 – 90)	82.5 (60 – 90)	86.7 (80 – 90)
Blood chemistry				
AST (U/l)	29.0 (25 – 32)	28.8 (26 – 32)	31.0 (25 – 37)	41.7 (18 – 90)
ALT (U/l)	24.3 (12 – 35)	21.3 (12 – 29)	45.0 (21 – 61)	20.3 (17 – 26)
Bilirubin (mg/dl)				
Direct	0.23 (0.2 – 0.3)	0.23 (0.2 – 0.3)	0.30 (0.2 – 0.5)	0.62 (0.1 – 2.8)
Total	0.57 (0.5 – 0.7)	0.50 (0.3 – 0.7)	0.65 (0.4 – 1)	1.19 (0.3 – 3.6)
Creatinine (mg/dl)	1.2 (0.8 – 1.6)	0.97 (0.7 – 1.58)	0.78 (0.5 – 1.2)	0.78 (0.6 – 1.0)
BUN (mg/dl)	16.6 (12.4 – 23.7)	16.8 (12.3 – 27.9)	15.9 (12.4 – 17.9)	14.3 (7.2 – 18.8)
Site of primary tumor in colon or rectum				
Ascending, *n* (%)	1 (33.3)	1 (25.0)	–	–
Transverse, *n* (%)	1 (33.3)	1 (25.0)	1 (25.0)	–
Descending, *n* (%)	1 (33.3)	1 (25.0)	2 (50.0)	1 (16.7)
Sigmoid	–	1 (25.0)	1 (25.0)	1 (16.7)
Rectosigmoid	–	–	–	2 (33.3)
Rectum	–	–	–	2 (33.3)
Site of metastasis				
Liver, *n* (%)	3 (100)	4 (100)	3 (75.0)	6 (100)
Bone, *n* (%)	2 (66.7)	2 (50.0)	1 (25.0)	–
Lymph node, *n* (%)	1 (33.3)	1 (25.0)	2 (50.0)	3 (50.0)
Peritoneum	–	1 (25.0)	2 (50.0)	–
Lung, *n* (%)	1 (33.3)	2 (50.0)	–	2 (33.3)
Primary tumor resection, *n* (%)	3 (100)	4 (100)	2 (50.0)	6 (100)
Earlier chemotherapy, *n* (%)	2 (66.7)	4 (100)	4 (100)	6 (100)
Earlier radiotherapy, *n* (%)	1 (33.3)	1 (25.0)	1 (25.0)	3 (50.0)

Figures are mean values or number of patients with ranges in brackets and percentages in parentheses.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, body surface area; BUN, blood urea nitrogen; HDFL, high-dose 5-fluorouracil and leucovorin; KPS, Karnofsky performance status.
[a]One patient had participated in trials of both I and II dosage levels.

Table 3 Pharmacokinetic parameters of 5-fluorouracil in patients with metastatic colorectal cancer at HDFL48 dosage levels I–III

	HDFL48 dosage levels (mg/48 h/week)			
PK parameters	I ($n=3$)	II ($n=4$[a])	III ($n=4$)	Total ($n=11$[a])
C_{SS} (μmol/l)	5.63 ± 3.61	4.74 ± 1.81	12.42 ± 8.24	7.78 ± 6.14
C_{BM} (μmol/l)	2.90 ± 1.14	2.06 ± 0.67	3.50 ± 0.84	2.89 ± 1.01
k (min^{-1})	0.104 ± 0.012	0.103 ± 0.051	0.115 ± 0.056	0.108 ± 0.041
$t_{1/2}$ (min)	6.72 ± 0.77	7.76 ± 3.27	7.16 ± 3.29	7.21 ± 2.51
Cl (ml/min)	3.284 ± 3.283	3.147 ± 1.587	1.696 ± 973	2.656 ± 1.944
V_d (l)	29.6 ± 26.5	39.4 ± 24.0	14.7 ± 6.8	26.6 ± 20.5

Results are given as mean ± standard deviation (SD).
C_{BM}, 5-fluorouracil BM concentration at the steady state, and concentration at the end of infusion; Cl, plasma clearance = infusion rate/C_{SS}; C_{SS}, 5-fluorouracil plasma concentration at the steady state, and concentration at the end of infusion; HDFL, high-dose 5-fluorouracil and leucovorin; k, terminal-phase rate constant, slope of semi-log plot of C_{SS}, C_{20}, C_{40} vs. time; PK, pharmacokinetic; $t_{1/2}$, terminal half-life = 0.693/k; V_d, apparent volume of distribution = Cl/k.
[a]One patient had participated trials of both I and II dosage levels.

Table 4 Comparison of 5-fluorouracil concentrations in plasma and bone marrow at steady state, levels I, II, and III

	5-FU concentrations at steady state (μmol/l)		P value[a]
Plasma (C_{SS}) ($n=10$)	8.06 ± 6.39	(1.58 – 23.69)	0.0210
Bone marrow (C_{BM}) ($n=10$)	2.89 ± 1.01	(1.39 – 4.73)	
C_{SS}-to-C_{BM} ratio	2.63 ± 1.40	(0.73 – 5.01)	
HDFL48 dosage level			
I ($n=3$)	2.02 ± 1.53	(0.73 – 3.71)	
II ($n=3$)	2.26 ± 0.89	(1.72 – 3.29)	
III ($n=4$)	3.38 ± 1.59	(1.92 – 5.01)	

Results are given as mean ± standard deviation with ranges in brackets.
C_{BM}, 5-fluorouracil (5-FU) BM concentration at the steady state, and concentration at the end of infusion; C_{SS}, 5-FU plasma concentration at the steady state, and concentration at the end of infusion; HDFL, high-dose 5-fluorouracil and leucovorin.
[a]t-test.

recommended dose of 5-FU was 3500 mg/m^2/48 h/week, with a fixed dose of LV at 300 mg/m^2/48 h/week in this HDFL48 trimonthly 48-h infusion schedule.

According to the protocol, the patients were allocated to HDFL48 for assessing 5-FU PK dispositions under dosage levels of I, II, and III. The measured steady-state C_{SS} and C_{BM} of 5-FU at various dosage levels are shown in Table 3. The mean values of C_{SS} and C_{BM} reached statistical difference (8.06 ± 6.39 vs. 2.89 ± 1.01 μmol/l, $P = 0.0210$; Table 4), with a mean C_{SS}-to-C_{BM} ratio of 2.63 ± 1.40. In fact, the C_{SS}-to-C_{BM} ratios were all above 1 (range, 1.61–5.01), with the exception of one particular dose-escalated individual, whose C_{SS}-to-C_{BM} ratio was 0.73 for dosage level I and was unknown (C_{BM} undetectable) for level II. The higher the dosage level, a tendency for higher C_{SS}-to-C_{BM} ratios was also noticed.

Other parameters, such as k, $t_{1/2}$, Cl, and V_d, were also calculated (Table 3). The $t_{1/2}$ was 7.21 ± 2.51 min and did not show much variation across the three dosage levels. In contrast, plasma clearance showed a large variance among individuals (range 592–7074 ml/min).

A multivariate analysis was done to delineate factors associated with chemotherapeutic clearance. However, the resulting data were not satisfactory ($r = 0.78$) and P values for variables such as age, sex, body weight, body surface area, Karnofsky performance status, and 5-FU dosage were all insignificant. Other factors that influence 5-FU PKs remain to be identified.

The correlation between the PK and pharmacodynamic effects of HDFL48, including efficacy and toxicity profiles, was further evaluated. Among the 16 intent-to-treat patients (including one patient who was nonevaluable for response, and another one who was chemonaive), three had objective partial responses [PRs; RR of 18.8%, 95% confidence interval (CI): 4–46%]; and two out of 14 patients who had failed HDFL24 (2600 mg/m^2/24 h/week) still had PRs to trimonthly HDFL48 (RR of 14.3%, 95% CI: 2–43%). Three responders were evenly distributed in the three dosage levels (one each in level I, II, and III, respectively). Median PFS was 4.1 months (range: 1.8–12.5 months). Median overall survival was 10.5 months (range: 2.7–32.1 months).

Major toxicities observed during the first cycle of HDFL48 therapy were selected for comparison analysis. Patients with hematological adverse reactions (leucopenia, anemia, thrombocytopenia) had significantly higher steady-state 5-FU BM concentrations (3.89 ± 1.04 vs. 2.46 ± 0.67 μmol/l, $P = 0.0279$; Table 5). Despite this difference, there was no significant difference in C_{SS} and C_{BM} levels between patients with and without mucositis or diarrhea ($P > 0.05$). Differences in C_{SS} and C_{BM} levels for HDFL48 responders ($C_{SS} = 7.28 \pm 5.94$ μmol/l, $C_{BM} = 2.42 \pm 0.52$ μmol/l) and nonresponders ($C_{SS} = 8.40 \pm 7.00$ μmol/l, $C_{BM} = 3.09 \pm 1.13$ μmol/l) were also not contributory ($P > 0.05$). A total of 80 trimonthly HDFL48 cycles were given to 16 patients with a median of four cycles (range: 1–12) per patient. It is worthy of mention that all toxicities were mainly of minor grade (Table 6). The most common toxicities were mucositis and diarrhea. Grade 1, 2, and 3 mucositis was noted in 33.8, 17.5, and 5.0% of cycles, respectively. Grade 1, 2, and 3 diarrhea was noted in 35.0, 8.8, and 1.3% of cycles, respectively. No grade IV toxicities were observed during the study. There were also no incidences of 5-FU-related hyperammonemic encephalopathy [28,30], neutropenic sepsis, or chemo therapy-related deaths.

Discussion

This phase I and PK studies assessed the PK dispositions of the HDFL48 regimen in patients with metastatic CRC. 5-FU dosages included 2500, 3000, 3500, and 3750 mg/m^2/48 h/week, with a flat dosage of LV administered at 300 mg/m^2/48 h/week. The MTD of 5-FU was 3750 mg/m^2/48 h/week. Although tumor response was not the 'primary' objective for this phase I study, objective PR was seen in 18.8% (95% CI: 4–46%) of intent-to-treat patients with tolerable adverse reactions.

Our earlier results by colony-forming unit-granulocyte and monocyte clonogenic assay showed that 24-h exposure to 5-FU (2 μmol/l) and 30-min exposure to 5-FU (100 μmol/l)

Table 6 Toxicity of the trimonthly HDFL48 regimen

	Cycles (n=80)			
Toxicity	Grade I (%)	Grade II (%)	Grade III (%)	Grade IV
Hematological				
Neutropenia	7.5	2.5	1.3	0
Leucopenia	10.0	15.0	1.3	0
Anemia	15.0	10.0	1.3	0
Thrombocytopenia	0	0	3.8	0
Fever	6.3	0	0	0
Infection	1.3	1.3	0	0
Gastrointestinal				
Nausea	7.5	1.3	0	0
Vomiting	13.8	2.5	0	0
Diarrhea	35.0	8.8	1.3	0
Mucositis	33.8	17.5	5.0	0
Hepatic	1.3	1.3	0	0
Neuropathy	0	0	0	0
Others				
Hand–foot syndrome	12.5	15.0	2.5	–
Alopecia	22.5	3.8	–	–

HDFL, high-dose 5-fluorouracil and leucovorin.

Table 5 Correlation of 5-fluorouracil concentrations with toxicity profiles under HDFL48 therapy

Toxicity or not			5-FU concentrations at steady state (μmol/l)			
			Plasma (C_{SS})		Bone marrow (C_{BM})	
Toxicity[a]	Yes/No	Number of patients	mean ± SD	P value	mean ± SD	P value
Hematology	+	3	11.76 ± 10.37	0.2524	3.89 ± 1.04	0.0279
	–	7	6.47 ± 3.95		2.46 ± 0.67	
Mucositis	+	7	7.94 ± 7.38	0.9325	2.70 ± 1.09	0.4078
	–	3	8.35 ± 4.52		3.32 ± 0.79	
Diarrhea	+	5	7.04 ± 3.99	0.6419	2.54 ± 0.79	0.3098
	–	5	9.08 ± 8.56		3.23 ± 1.17	

Results are given as mean ± standard deviation (SD).
C_{BM}, 5-fluorouracil (5-FU) BM concentration at the steady state, and concentration at the end of infusion; C_{SS}, 5-FU plasma concentration at the steady state, and concentration at the end of infusion; HDFL, high-dose 5-fluorouracil and leucovorin; SD, standard deviation.
[a]Main toxicities recorded during the first cycle of HDFL48 therapy.

resulted in 27.2 and 78.2% inhibition of colony-forming unit-granulocyte and monocyte, respectively [19]. These in-vitro data provided direct evidence that may explain why myelotoxicity is significantly less during weekly 24-h infusion of 5-FU than is reported to occur after conventional bolus regimens [19]. Moreover, this 48-h 5-FU continuous-infusion study confirmed that 5-FU concentrations that are relatively high in plasma and low in BM might further minimize myelotoxicity. The dosage range used in this trimonthly HDFL48 regimen achieved an average 2.63-fold concentration gradient between plasma and BM compartments at steady-state concentrations. The observation of higher C_{SS}-to-C_{BM} ratios at elevated dosage levels might indicate that 5-FU is compartmentalized discretely in the plasma and BM under the tested dosages. A significant association between BM concentrations of 5-FU and hematological toxicity was also shown (3.89 ± 1.04 vs. 2.46 ± 0.67 μmol/l, $P = 0.0279$; Table 5). Fraile *et al.* [13] first reported that 96-h intravenous infusions of 5-FU in six patients resulted in constant levels of 5-FU in plasma and significantly less 5-FU in BM. The investigators suggested that the route of the administration-dependent PK profile is consistent with the reported absence of myelosuppression in 96-h infusion and may be related to the resultant lower levels of 5-FU achieved in BM. To the best of our knowledge, this study is the first to investigate the BM (C_{BM}) concentrations of 5-FU with this 48-h 5-FU Ci regimen using dosage escalations subsequent to the report by Fraile *et al.* [13].

Early in-vitro data have shown that the minimal cytotoxic concentration of 5-FU for tumor cells was 0.38 μmol/l [31]. The HDFL48 protocol yielded a mean C_{SS} of 7.78 μmol/l, approximately 20-fold higher than the abovementioned effective concentration. This adequately high plasma 5-FU level sustained for 48 h might lead to superior clinical responses than are observed with bolus schedules (which usually result in sustained levels for less than 1 h; that is, four times the 5-FU half-life) or the popular HDFL24 (sustained levels for 24 h). In fact, two out of 14 patients who had failed earlier HDFL24 treatment experienced a PR (RR of 14.3, 95% CI: 2–43%) with trimonthly HDFL48. Thus, HDFL48 could be used as an important component of salvage chemotherapy.

Tournigand *et al.* [5] reported pivotal data from a randomized phase III study of FOLFIRI followed by FOLFOX6 or the reverse sequence (FOLFOX6 followed by FOLFIRI) in patients with advanced CRC. FOLFIRI consists of a 2-h infusion of l-LV (200 mg/m^2) or dl-LV (400 mg/m^2), followed by a FU bolus of 400 mg/m^2 and a 46-h infusion of 2400–3000 mg/m^2 every 46 h every 2 weeks, with irinotecan (180 mg/m^2) as a 2-h infusion on day 1. FOLFOX6 consists of a 2-h infusion of l-LV (200 mg/m^2) or dl-LV (400 mg/m^2), followed by a FU bolus of 400 mg/m^2 and a 46-h infusion of 2400–3000 mg/m^2 every 46 h every 2 weeks, with oxaliplatin (100 mg/m^2) as a 2-h infusion on day 1. In the setting of salvage chemotherapy for metastatic CRC, second-line FOLFIRI achieved a 4% RR and a median of 2.5 months of PFS, whereas second-line FOLFOX6, which achieved a 15% RR and 4.2 months of PFS [5]. In this phase I study, trimonthly HDFL48 achieved a 14.3% RR (95% CI: 2–43%) and a median of 4.1 months of PFS with minimal toxicity, showing its potential as an important component in salvage chemotherapy.

Adverse reactions associated with HDFL48 were not significantly different from reactions reported earlier for HDFL24 protocols [21,23]. In particular, 5-FU-related hyperammonemic encephalopathy [28,30,32] was not seen in this study. Between 1991 and 1995, 16 patients (5.7%) developed HDFL-related encephalopathy among the 280 patients who had received HDFL in our institution [28]. Among a series of biochemical and hematological parameters examined, the serum triglyceride level was the only key predictive maker for HDFL-related hyperammonemic encephalopathy. The serum triglyceride level of the patients who developed encephalopathy was significantly lower than that of those patients who did not ($P < 0.001$ by Wilcoxon rank-sum test) [28]. Since then, the fasting serum triglyceride of more than or equal to 70 mg/dl has been added to the inclusion criteria of all of the future prospective clinical trials for HDFL-based chemotherapy. The incidence of HDFL-related hyperammonemic encephalopathy has been markedly decreased to less than 1% (unpublished data). Overall, this favorable finding was probably attributable to the exclusion of patients with hypotriglyceridemia based on earlier experiences [28,30] and a longer infusion schedule resulting in the attainment of adequate and sustained 5-FU plasma levels without a reduction in the overall 5-FU doses. The HDFL48 regimen would, therefore, be a reasonably favorable choice for use in combination with other agents (such as irinotecan, oxaliplatin, bevacizumab, or cetuximab) to improve RRs and still minimize toxicity.

The apparent half-life of 5-FU in this 48-h continuous-infusion cohort ranged from 4.3 to 11.6 min, which was comparable with the results reported for other studies [4]. Intersubject variability in 5-FU PK parameters, especially the C_{SS}, Cl, and V_d (Table 3), was evident within the same dosage schedule and among different dosage levels as described earlier [9]. A multitude of factors including genetic traits [33–35] have been recognized as having an impact on 5-FU PKs and subsequently its pharmacodynamics. It is not surprising that the variability in PK characteristics was also noted in this study and that the C_{SS} and Cl values were not predictive of myelosuppression or gastrointestinal toxicities. Nonetheless, this small patient cohort, complicated disease state, limited blood-sampling schema, utilization of a linear PK model, and lack of genetic screening may all contribute in part to this substantial variance.

It is estimated that one-fourth of the patients with CRC present with overt metastases at the time of initial diagnosis. Undoubtedly, surgical resection, in addition to 5-FU-based pharmacotherapy, will remain the mainstay of treatment for advanced CRC. Prudent selection and stratification of patients based on their characteristics [36], blood biochemistry [28,37], gene expression signature [38], and PK-guided dosage adjustment [16,17] should evolve to optimize CRC pharmacotherapy in the years to come.

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References

1 Coleman MP, Quaresma M, Berrino F, Lutz JM, De Angelis R, Capocaccia R, *et al.* Cancer survival in five continents: a worldwide population-based study (CONCORD). *Lancet Oncol* 2008; **9**:730–756.
2 Heidelberger C, Chaudhuri NK, Danneberg P, Mooren D, Griesbach L, Duschinsky R, *et al.* Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature* 1957; **179**:663–666.
3 Meta-analysis Group in Cancer. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. *J Clin Oncol* 1998; **16**:301–308.
4 Grem JL. 5-fluoropyrimidines. In: Chabner BA, Longo DL, editors. *Cancer chemotherapy and biotherapy: principles and practice*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. pp. 125–182.
5 Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, *et al.* FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**:229–237.
6 Falcone A, Ricci S, Brunetti I, Pfanner E, Allegrini G, Barbara C, *et al.* Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. *J Clin Oncol* 2007; **25**:1670–1676.
7 Ramanathan RK, Bjarnason GA, Bernard SA, Desimone P, Braich T, Evars JP, *et al.* A four-arm, randomized, multicenter phase II trial of oxaliplatin combined with varying schedules of 5-fluorouracil as first-line therapy in previously untreated advanced colorectal cancer. *Clin Colorectal Cancer* 2008; **7**:134–139.
8 Saunders M, Iveson T. Management of advanced colorectal cancer: state of the art. *Br J Cancer* 2006; **95**:131–138.
9 Takimoto CH, Yee LK, Venzon DJ, Schuler B, Grollman F, Chabuk C, *et al.* High inter- and intrapatient variation in 5-fluorouracil plasma concentrations during a prolonged drug infusion. *Clin Cancer Res* 1999; **5**:1347–1352.
10 Shah A, MacDonald W, Goldie J, Gudauskas G, Brisebois B. 5-FU infusion in advanced colorectal cancer: a comparison of three dose schedules. *Cancer Treat Rep* 1985; **69**:739–742.
11 Hryniuk WM, Figueredo A, Goodyear M. Applications of dose intensity to problems in chemotherapy of breast and colorectal cancer. *Semin Oncol* 1987; **14** (**4 Suppl 4**):S3–S11.
12 De Gramont A, Bosset JF, Milan C, Rougier P, Bouche O, Etienne PL, *et al.* Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. *J Clin Oncol* 1997; **15**:808–815.
13 Fraile RJ, Baker LH, Buroker TR, Horwitz J, Vaitkevicius VK. Pharmacokinetics of 5-fluorouracil administered orally, by rapid intravenous and by slow infusion. *Cancer Res* 1980; **40**:2223–2228.
14 Beerblock K, Rinaldi Y, Andre T, Louvet C, Raymond E, Tournigand C, *et al.* Bimonthly high dose leucovorin and 5-fluorouracil 48-h continuous infusion in patients with advanced colorectal carcinoma. Groupe d'Etude et de Recherche sur les Cancers de l'Ovaire et Digestifs (GERCOD). *Cancer* 1997; **79**:1100–1105.
15 Aranda E, Diaz-Rubio E, Cervantes A, Anton-Torres A, Carrato A, Massuti T, *et al.* Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with weekly high-dose 48-h continuous infusion fluorouracil for advanced colorectal cancer: a Spanish Cooperative Group for Gastrointestinal Tumor Therapy (TTD) study. *Ann Oncol* 1998; **9**:727–731.
16 Gamelin E, Boisdron-Celle M, Delva R, Regimbeau C, Cailleux PE, Alleaume C, *et al.* Long-term weekly treatment of colorectal metastatic cancer with fluorouracil and leucovorin: results of a multicentric prospective trial of fluorouracil dosage optimization by pharmacokinetic monitoring in 152 patients. *J Clin Oncol* 1998; **16**:1470–1478.
17 Gamelin E, Delva R, Jacob J, Merrouche Y, Raoul JL, Pezet D, *et al.* Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 2099–2105.
18 Yeh KH, Yeh SH, Hsu CH, Wang TM, Ma IF, Cheng AL. Prolonged and enhanced suppression of thymidylate synthase by weekly 24-h infusion of high-dose 5-fluorouracil. *Br J Cancer* 2000; **83**:1510–1515.
19 Yeh KH, Yeh SH, Chang YS, Cheng AL. Minimal toxicity to myeloid progenitor cells of weekly 24-h infusion of high-dose 5-fluorouracil: direct evidence from colony-forming unit granulocyte and monocyte (CFU-GM) clonogenic assay. *Pharmacol Toxicol* 2000; **86**:122–124.
20 Spicer DV, Ardalan B, Daniels JR, Silberman H, Johnson K. Reevaluation of the maximum tolerated dose of continuous venous infusion of 5-fluorouracil with pharmacokinetics. *Cancer Res* 1988; **48**:459–461.
21 Ardalan B, Chua L, Tian EM, Reddy R, Sridhar K, Benedetto P, *et al.* A phase II study of weekly 24-h infusion with high-dose fluorouracil with leucovorin in colorectal carcinoma. *J Clin Oncol* 1991; **9**:625–630.
22 Yeh KH, Cheng AL. An alternative method to overcome central venous portable external infusion pump blockage in patients receiving weekly 24-h high-dose fluorouracil and leucovorin. *J Clin Oncol* 1994; **12**:875–876.
23 Yeh KH, Cheng AL, Lin MT, Hong RL, Hsu CH, Lin JF, *et al.* A phase II study of weekly 24-h infusion of high-dose 5-fluorouracil and leucovorin (HDFL) in the treatment of recurrent or metastatic colorectal cancers. *Anticancer Res* 1997; **17**:3867–3871.
24 Diaz-Rubio E, Aranda E, Martin M, Gonzalez-Mancha R, Gonzalez-Larriba J, Barneto I. Weekly high-dose infusion of 5-fluorouracil in advanced colorectal cancer. *Eur J Cancer* 1990; **26**:727–729.
25 Aranda E, Cervantes A, Carrato A, Fernandez-Martos C, Anton-Torres A, Massuti T, *et al.* Outpatient weekly high-dose continuous infusion 5-fluorouracil plus oral leucovorin in advanced colorectal cancer. A phase II trial. Spanish Cooperative Group for Gastrointestinal Tumor Therapy (TTD). *Ann Oncol* 1996; **7**:581–585.
26 Aranda E, Cervantes A, Dorta J, Blanco E, Fernandez-Martos C, Cruz-Hernandez JJ, *et al.* A phase II trial of weekly high dose continuous infusion 5-fluorouracil plus oral leucovorin in patients with advanced colorectal cancer. The Spanish Cooperative Group for Gastrointestinal Tumor Therapy (TTD). *Cancer* 1995; **76**:559–563.
27 De Gramont A, Louvet C, Andre T, Tournigand C, Krulik M. A review of GERCOD trials of bimonthly leucovorin plus 5-fluorouracil 48-h continuous infusion in advanced colorectal cancer: evolution of a regimen. Groupe d'Etude et de Recherche sur les Cancers de l'Ovaire et Digestifs (GERCOD). *Eur J Cancer* 1998; **34**:619–626.
28 Yeh KH, Cheng AL. High-dose 5-fluorouracil infusional therapy is associated with hyperammonaemia, lactic acidosis and encephalopathy. *Br J Cancer* 1997; **75**:464–465.
29 Escoriaza J, Aldaz A, Calvo E, Giraldez J. Simple and sensitive determination of 5-fluorouracil in plasma by high-performance liquid chromatography. Application to clinical pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 1999; **736**:97–102.
30 Yeh KH, Cheng AL. 5-fluorouracil-related encephalopathy: at least two distinct pathogenetic mechanisms exist. *Br J Cancer* 1998; **77**: 1711–1712.
31 Reeves WJ Jr, Cailleau R. Mechanism of growth inhibition by 5-fluorouracil: reversal studies with pyrimidine metabolites *in vitro*. *Proc Soc Exp Biol Med* 1969; **131**:1068–1072.
32 Yeh KH, Cheng AL. Acute confusion induced by a high-dose infusion of 5-fluorouracil and folinic acid. *J Formos Med Assoc* 1994; **93**:721–723.
33 Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, Sherrod A, *et al.* Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics J* 2001; **1**:65–70.

34 Fernandez-Contreras ME, Sanchez-Prudencio S, Sanchez-Hernandez JJ, Garcia de Paredes ML, Gisbert JP, Roda-Navarro P, *et al.* Thymidylate synthase expression pattern, expression level and single nucleotide polymorphism are predictors for disease-free survival in patients of colorectal cancer treated with 5-fluorouracil. *Int J Oncol* 2006; **28**:1303–1310.
35 Yen JL, McLeod HL. Should DPD analysis be required prior to prescribing fluoropyrimidines? *Eur J Cancer* 2007; **43**:1011–1016.
36 Sorbye H, Kohne CH, Sargent DJ, Glimelius B. Patient characteristics and stratification in medical treatment studies for metastatic colorectal cancer: a proposal for standardization of patient characteristic reporting and stratification. *Ann Oncol* 2007; **18**:1666–1672.
37 Fountzilas G, Gossios K, Zisiadis A, Svarna E, Skarlos D, Pavlidis N. Prognostic variable in patients with advanced colorectal cancer treated with fluorouracil and leucovorin-based chemotherapy. *Med Pediatr Oncol* 1996; **26**:305–317.
38 Del Rio M, Molina F, Bascoul-Mollevi C, Copois V, Bibeau F, Chalbos P, *et al.* Gene expression signature in advanced colorectal cancer patients select drugs and response for the use of leucovorin, fluorouracil, and irinotecan. *J Clin Oncol* 2007; **25**:773–780.