Cancer Chemother Pharmacol (2008) 61:579–585
DOI 10.1007/s00280-007-0509-5

ORIGINAL ARTICLE

A phase I and pharmacokinetic study of OSI-7904L, a liposomal thymidylate synthase inhibitor in combination with oxaliplatin in patients with advanced colorectal cancer

A. R. Clamp · P. Schöffski · J. W. Valle · R. H. Wilson · S. Marreaud · A.-S. Govaerts · M. Debois · D. Lacombe · C. Twelves · J. Chick · G. C. Jayson · on behalf of the EORTC New Drug Development Group

Received: 26 February 2007 / Accepted: 22 April 2007 / Published online: 23 May 2007

Abstract
Purpose OSI-7904L is a liposomal formulation of a potent thymidylate synthase (TS) inhibitor. This phase I study evaluated the safety, tolerability and pharmacokinetics (PK) of OSI-7904L administered in combination with oxaliplatin every 21 days in patients with advanced colorectal carcinoma.
Method A 3+3 study design was utilized at predefined dose levels. Polymorphisms in the TS enhancer region and XPD enzyme were investigated as potential predictors of efficacy and toxicity.
Results Fourteen patients received 76 cycles of treatment. At the highest dose level (OSI-7904L 9 mg/m^2, oxaliplatin 130 mg/m^2) investigated, one of nine patients experienced dose-limiting toxicity of grade 3 oral mucositis with cycle 1 and five further patients required dose reductions. The toxicity profile of stomatitis, diarrhea, nausea, fatigue, sensory neuropathy and skin rash was consistent with that expected for a TS inhibitor/oxaliplatin combination regimen. PK analysis showed high interpatient variability with no detectable interaction between OSI-7904L and oxaliplatin. Partial radiological responses were documented in two patients.
Conclusions The recommended regimen for further investigation is OSI-7904L 9 mg/m^2 and oxaliplatin 130 mg/m^2.

Keywords Phase I · OSI-7904L · Oxaliplatin · Thymidylate synthase · Colorectal carcinoma

A. R. Clamp (✉) · J. W. Valle · G. C. Jayson
Cancer Research UK and University of Manchester Department of Medical Oncology, Christie Hospital, Wilmslow Road, Manchester M20 4BX, UK
e-mail: Andrew.Clamp@christie.nhs.uk

P. Schöffski
Department of Hematology, Hemostaseology and Oncology, Hannover Medical School, Hannover, Germany

R. H. Wilson
Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast BT9 7AB, UK

S. Marreaud · A.-S. Govaerts · M. Debois · D. Lacombe · C. Twelves
EORTC New Drug Development Program (NDDP), Brussels, Belgium

J. Chick
OSI Pharmaceuticals Inc, Oxford, UK

Introduction

For many years, the standard of care for metastatic colorectal carcinoma (CRC) has been 5-fluorouracil (5-FU) -based chemotherapy [1–4]. 5-FU acts mainly as an inhibitor of thymidylate synthase (TS) [5]. However, several clinically relevant 5-FU resistance mechanisms can develop and prolonged infusional regimens are required for optimal efficacy [6]. Much research has focused therefore on the development of newer TS inhibitors [7, 8].

OSI-7904L is the liposomal formulation of OSI-7904 [(S)-2-[5-[1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl)methyl]amino-1-oxo-2-isoindolynl]-glutaric acid], a potent selective non-competitive TS inhibitor. It consists of small (20–80 nm) unilamellar vesicles containing OSI-7904 within their aqueous cores. Pre-clinical studies in murine CRC xenograft models have demonstrated superior antitumour efficacy for OSI-7904L compared to OSI-7904 and 5-FU and liposomal encapsulation greatly increases plasma, tissue and tumor exposure to OSI-7904 [9]. A phase I study

evaluated OSI-7904L administered intravenously every 3 weeks in patients with advanced cancer [10]. It established a recommended single agent dose of 12 mg/m^2 with a toxicity profile consisting of rash, pruritus, lethargy, stomatitis and myelosuppression. Although no responses were documented, prolonged disease stabilization was noted in 7 of 22 patients with CRC, all of whom had received previous TS inhibitors. Pharmacokinetic analysis confirmed that liposomal encapsulation increased plasma residence time.

Clinical data indicating improved outcomes with 5-FU/oxaliplatin regimens [3, 4] and non-overlapping toxicity profiles provide a strong rationale to evaluate the combination of OSI-7904L and oxaliplatin. This is supported by preclinical data, which indicate that the activity of the OSI-7904L/ oxaliplatin combination is at least additive [11]. Work with the COLO 205 xenograft indicated improved efficacy when oxaliplatin was administered 24 h prior to OSI-7904L compared to after, suggesting potential schedule dependence. This is supported by in vivo research in oesophageal carcinomas which indicates that TS expression is downregulated after oxaliplatin exposure [12].

Pharmacogenomic studies have identified genetic polymorphisms that may allow the selection of patients most likely to benefit from specific cytotoxic agents. TS expression is modulated by the number of 28 base pair tandem repeats within the 5′-untranslated enhancer region. Individuals homozygous for a triple repeat (3R) have elevated levels of TS protein and this may be associated with worse clinical outcome in CRC [13, 14]. A G-to-C single nucleotide polymorphism within the second repeat of 3R alleles may abolish this increased TS expression and also influence sensitivity to TS inhibitors [15, 16]. Correspondingly, it has been suggested that polymorphisms in the XPD enzyme, part of the nucleotide excision repair pathway, may affect sensitivity to platinum-based chemotherapy [17, 18]. A recent retrospective study has indicated that homozygosity for *Lys*/*Lys* at the *Lys*751*Gln* polymorphism is associated with a poorer outcome in patients with heavily pretreated CRC given oxaliplatin/5-FU [19].

In this phase I study, we aimed to establish the safety, toxicity profile and recommended doses of oxaliplatin and OSI-7904L administered on day 1 of a 21-day cycle. Secondary objectives included assessment of the plasma pharmacokinetic profile of oxaliplatin, free and liposomal OSI-7904, antitumour activity and an exploratory pharmacogenetic analysis.

Patients and methods

Patient selection

Eligible patients had histologically documented CRC and measurable radiological evidence of advanced disease. One prior line of non-oxaliplatin containing chemotherapy for advanced disease was permitted provided that subsequent disease progression had been documented. Other eligibility criteria included age ≥18; ECOG performance status ≤2; recovery from previous treatment-related toxicities; no anticancer therapy for ≥3 weeks; adequate haematopoietic (neutrophils > 1.5×10^9/L, platelet count >100×10^9/L), hepatic (bilirubin < 1.5 times upper limit of normal (ULN), aspartate and alanine aminotransferases <2.5 times ULN in the absence of liver metastases and <5 times ULN in the presence if liver metastases) and renal (creatinine <1.5 times ULN) function; no pre-existing neuropathy ≥CTC grade 2; no known hypersensitivity to liposomal formulations; no known cerebral metastases or serious concomitant medical conditions.

The study was approved by ethical committees at all participating centers. All patients gave written informed consent prior to study entry.

Treatment and dose escalation

Oxaliplatin and OSI-7904L were administered on day 1 every 21 days. Oxaliplatin was administered in 250–500 ml 5% dextrose as an intravenous infusion over 2 h. OSI-7904L was diluted in 5% dextrose and administered as an intravenous infusion over 30 min, commencing 30 min after completion of oxaliplatin due to preclinical data suggesting schedule dependence [11].

Dose escalation proceeded in a stepwise manner with four planned dose levels (dose level 1- OSI-7904L 6 mg/m^2, oxaliplatin 100 mg/m^2; dose level 2- 6/130; dose level 3- 9/130; dose level 4- 12/130). Dose escalation was based on safety assessment of the previous cohort using a 3+3 design. If no patients experienced a dose-limiting toxicity (DLT) during the first treatment cycle, dose escalation was permitted. If 1 of 3 patients experienced a DLT up to 3 more patients were recruited at that dose level. Maximum tolerated dose (MTD) was defined as the highest dose at which ≤1 DLT was observed among six patients during the first treatment cycle.

Toxicities were graded using National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 [20]. DLTs were defined as; grade 3–4 non hematological toxicity except nausea, vomiting, diarrhea, rash or infusion related reaction rapidly controlled with appropriate measures; grade 3 elevation of alanine or aspartate aminotransferase levels lasting >7 days; altered cardiac function ≥grade 2; absolute neutrophil count (ANC) <0.5×10^9/L lasting ≥7 days; febrile neutropenia (ANC < 1.0×10^9/L and fever ≥ 38.5°C); grade 3 or 4 infection with ANC <1.0×10^9/L; thrombocytopenia ≤ 25×10^9/L or thrombocytopenic bleeding requiring transfusion.

Treatment was repeated every 21 days and could continue for up to 6 cycles in the absence of disease progression. Treatment beyond six cycles was allowed at the sponsor's discretion (OSI Pharmaceuticals, Oxford, UK).

Dose modifications

A single dose reduction to the next lower dose level was permitted if a patient experienced DLT or other significant toxicity. No dose re-escalation was allowed. If toxicity was felt to be related to one drug, the dose of that drug could be reduced alone at the treating investigators discretion.

Study investigations

Baseline assessments were performed within two weeks of trial treatment. These included history, physical examination, ECOG performance status (PS), electrocardiograph, complete blood count (CBC) and serum biochemistry. Tumour evaluation was performed by CT scanning. During treatment, physical examination, PS and CBCs were repeated weekly. Serum biochemistry was checked weekly during the first cycle and thereafter at the end of each cycle. Tumour response was assessed after every two cycles using RECIST criteria [21]. Patients whose disease progressed were taken off study.

Plasma pharmacokinetics

Pharmacokinetic sampling took place during cycle 1 at the following timepoints; predose, 2, 2.5, 3, 4, 7, 24, 48, 96, 168 h and days 15 and 22 after start of oxaliplatin infusion. OSI-7904 pharmacokinetics samples were collected into EDTA tubes, cooled immediately on ice and then centrifuged at 1,500*g* for 10 min under refrigeration. Two 0.5 ml aliquots were stored at −20°C and analysed to determine total plasma OSI-7904. The remaining plasma was then split between the reservoirs of two Centifree® micropartition devices (Amicon) and centrifuged at 1,500*g* for 10 min under refrigeration. The collected ultrafiltrate was pooled and stored at −20°C for analysis of free plasma OSI-7904. Blood samples for oxaliplatin pharmacokinetics were collected into heparin containing vials, cooled immediately on ice and centrifuged under refrigeration for 10 min at 1,500*g*. The plasma was then transferred to the reservoirs of 2 Centrifree® devices and centrifuged at 1,500*g* for 30 min under refrigeration. The ultrafiltrate was pooled and stored at −20°C. Samples were shipped to MDS Pharma Services St-Laurent (Montreal, Quebec, Canada) for analysis by previously validated methods. Non-compartmental analysis of the data generated was performed using WinNonlin version 4.1 (Pharsight Corporation, Mountain View, CA). Model 202 (intravenous infusion) was applied with the linear/log trapezoidal rule.

TS and XPD polymorphisms

A 10 ml blood sample was collected into a vial containing EDTA, gently inverted and split into two 5 ml aliquots before storage at −20°C. Genomic DNA was purified using a Qiagen QIAamp® DNA blood kit and stored at −20°C until genotyping by previously described methods. TS genotype analysis [15] was performed at the University of Chicago Genetic Services Laboratory, Chicago, IL while XPD genotype analysis [18] was undertaken at OSI Pharmaceuticals Inc., Boulder, CO.

Results

Fifteen patients were entered at three centers. Median age was 61 years (range 51-72) and ten were male. Four patients were ECOG PS0, ten PS1 and one PS2. All patients had received prior TS inhibitors (3 adjuvant, 13 advanced). Nine patients had also received irinotecan and 3 palliative radiotherapy before trial entry.

Fourteen patients were evaluable for response and toxicity. One patient did not receive treatment due to rapid disease progression. Seventy-six cycles of OSI-7904L and oxaliplatin were administered (median = 6, range 0–10) over three dose levels

Toxicity

Mild treatment-related toxicities (Table 1) only were documented on dose levels one and two. At dose level 3 (OSI-7904L 9 mg/m^2, oxaliplatin 130 mg/m^2) one DLT was reported. This patient developed grade 3 oral mucositis associated with grade 2 diarrhea, grade 2 truncal skin desquamation and grade 4 neutropenia. After a one-week delay, the patient received three cycles at dose level 2 with mild cutaneous toxicity only prior to disease progression. Dose level three was subsequently expanded to eight patients without further DLTs. Further dose escalation was not undertaken following pharmacokinetic data review and emerging tolerability data from other concurrent studies [22].

Most treatment-related toxicities on dose level three were manageable although two patients discontinued treatment because of adverse events (one- recurrent acute grade 3 sensory neuropathy, one- worsening fatigue, anorexia and nausea). Six patients, including the patient experiencing DLT, treated on dose level three required dose reductions (two- hematologic toxicity, one- grade 3 oral mucositis, one- grade 3 diarrhoea, two- multiple grade two non-hematological toxicities interfering with quality of life). These reductions occurred after cycles 1, 2, 3, 5 and 5. There was no evidence for cumulative toxicity apart from fatigue (grade 2 in 6/8, 75%) and oxaliplatin-related cold-sensitive

Table 1 Haematologic and non-haematologic toxicity possibly related to study treatment by dose level (includes all events occurring in ≥10% patients or >CTC grade 2 across all cycles)

	Level 1		Level 2			Level3			
	(*n* = 3, 18 cycles)		(*n* = 3, 18 cycles)			(*n* = 8, 40 cycles)			
OSI-7904L (mg/m^2)	6		6			9			
Oxaliplatin (mg/m^2)	100		130			130			
No. DLTs	0		0			1			
No. TDs	0		0			2			
Toxicity grade	1	2	1	2	3	1	2	3	4
Fatigue	2	–	3	–	–	2	6	–	–
Fever	–	2	–	–	–	1	–	–	–
Skin-related	1	1	–	2	–	3	1	–	-
Anorexia	–	1	1	–	–	2	2	–	–
Constipation	–	–	–	–	–	2	1	–	–
Diarrhoea	2	–	1	1	–	3	3	1	–
Stomatitis	1	1	2	1	–	4	2	1	–
Nausea	1	2	2	–	–	3	4	–	–
Vomiting	1	2	–	1	–	2	1	–	–
Other GI	1	–	1	–	–	2	1	–	–
Motor Neuropathy	–	–	–	–	–	2	–	–	–
Sensory neuropathy	3	–	1	2	–	3	4	1	–
Neuro-other	–	–	1	–	–	1	2	–	–
Pain	1	–	1	–	–	2	–	–	–
Pulmonary	–	–	–	–	–	2	–	–	–
Haematologic toxicity									
Leukocytes	1	–	1	–	–	1	2	2	–
Neutrophils	–	–	–	–	–	1	–	1	2
Platelets	–	–	1	–	–	2	3	–	–
Haemoglobin	1	–	–	3	–	2	5	–	–

DLT dose limiting toxicity, *TD* treatment discontinuations for toxicity prior to cycle 6

sensory neuropathy, which increased from mild to moderate during treatment.

Dermatologic toxicities occurred in the majority of patients (8/14, 57%). Toxicities were generally confined to the trunk and lower limbs and consisted of erythematous/desquamative eruptions that resolved without specific treatment. No cases of palmo-plantar erythema were reported.

Anti-tumor activity

Two partial disease responses were reported. These occurred in one patient on dose level one who continued therapy for ten cycles before progressive disease and in one patient on dose level two who discontinued treatment after six cycles due to recurrent cutaneous toxicity. Both patients had received prior TS inhibitor treatment for advanced disease. Eleven of twelve other treated patients achieved disease stabilization as best disease response with four of eight patients on dose level three remaining stable at planned treatment completion.

Pharmacokinetics

Oxaliplatin and total and ultrafilterable (unencapsulated and non-protein bound) OSI-7904 plasma pharmacokinetics were calculated for 10 and 14 patients respectively (see Table 2). Substantial interpatient variability in OSI-7904 pharmacokinetics occurred as exemplified by the 29-fold range observed for plasma clearance (Table 2). The plasma terminal half-life was generally more consistent. For example, following the 9 mg/m^2 dose of OSI-7904L, the plasma terminal half-life ranged only 1.7-fold. Therefore the terminal half-life could not be responsible for the 29-fold range in the plasma clearance.

These data are consistent with the observation of Beutel et al. [10] that showed that the variability in clearance is due to the depth of the initial clearance phase.

Ultrafilterable OSI-7904L plasma concentrations were much lower than those of total OSI-7904L and were undetectable in 9/12 patients 21.5 h after initiation of OSI-7904L infusion. Ultrafilterable OSI-7904 C_{max} (median:range) were 1.48 (0.23–2.06) ng/ml and 2.74

Table 2 Pharmacokinetic variables following first cycle administration for OSI-7904L/ oxaliplatin. Variables recorded as median (range). Dose levels are pooled as no pharmacokinetic interactions between OSI-7904 and oxaliplatin were noted

OSI-7904 (total)					
Dose (mg/m^2)	*n*	C_{max} (μg/mL)	$AUC_{0-\infty}$ (h μg/mL)	CL (mL/h/m^2)	$T_{1/2}\lambda z$ (h)
6	6	2.10 (1.94–2.86)	60.0 (7.93–181)	38.5 (33.1–756)	66.5 (47.8–507)
9	8	3.67 (2.18–4.87)	97.0 (10.9–319)	92.8 (28.2–827)	65.6 (56.2–97.3)
Oxaliplatin[#]					
Dose (mg/m^2)	*n*	C_{max} (ng/ml)	AUC_{0-last} (h ng/mL)	CL (L/h/m^2)	$T_{1/2}\lambda z$ (h)
100	3	960 (819–1060)	4.19 (3.68–5.35)	–	16.6 (12.2–18.9)
130	7	1640 (802–2130)	9.85 (7.18–26.1)	5.01 (2.38–7.26)	34.1 (17.9–306)

[#] AUC_{0-last} reported as calculation of $AUC_{0-\infty}$ would have required extrapolation beyond AUC_{0-last} by more than 20% at 100 mg/m^2 dose level

(1.43–134) ng/ml at the 6 and 9 mg/m^2 dose levels. These are approximately 1400-fold lower than total OSI-7904L levels. Assuming 96% protein binding for free OSI-7904, extraliposomal plasma OSI-7904 levels are about 50-fold lower than encapsulated OSI-7904 concentrations.

Although the effects of oxaliplatin administration on subsequent OSI-7904L pharmacokinetics are difficult to determine due to the high interpatient variability, the median C_{max}, AUC and terminal half-lives are consistent with those obtained in single agent studies [10, 22]. Notably, no differences were seen between patients treated on dose levels 1 and 2 who all received 6 mg/m^2 OSI-7904L but either 100 or 130 mg/m^2 of oxaliplatin. This indicates that prior administration of oxaliplatin had little effect on OSI-7904L pharmacokinetics. Oxaliplatin pharmacokinetics are also consistent with previously reported data [23].

Pharmacogenomics

TS 5′-enhancer region (TSER) and XPD genetic polymorphisms were analyzed in the 14 patients who received protocol treatment. Analysis of both the number of tandem repeats and the SNP status of the TSER was undertaken. Two patients were 2RG/2RG, four were 2RG/3RG, three were 2RG/3RC, three were 3RG/3RC and 2 were 3RC/3RC. No patients carried the high TS expression 3RG/3RG genotype [13, 16]. No correlations were noted between repeat number and toxicity or disease response/stabilization.

Analysis of the XPD *Lys*751*Gln* polymorphism [17, 19] identified three patients who were *Lys*/*Lys*, nine were *Lys*/*Gln* and two *Gln*/*Gln*. Two of three patients carrying *Lys*/*Lys* progressed on treatment but these were treated at the lowest dose level. No correlation between XPD genotype and toxicity was noted.

In summary, no relationships between toxicity and/or efficacy of treatment and the candidate biomarkers assessed were apparent.

Discussion

This phase I trial has established the safety of combination therapy with OSI-7904L and oxaliplatin in patients with metastatic CRC. Although MTD was not formally reached, the toxicity data obtained in combination with results from other clinical trials investigating OSI-7904L [10, 22] indicate that the recommended three-weekly regimen for further investigation should be 130 mg/m^2 oxaliplatin infused over 2 h followed by 9 mg/m^2 OSI-7904L given over 30 min. This contains the single-agent MTD for 3-weekly oxaliplatin [24] combined with OSI-7904L at 75% single-agent MTD [10, 22].

The toxicity profile observed is consistent with other TS inhibitor/oxaliplatin combinations [4, 25]. Predominant toxicities were gastrointestinal (stomatitis, diarrhea and nausea), cutaneous, fatigue and sensory neuropathy. Grade 3 stomatitis (DLT), diarrhoea and sensory neuropathy were each seen in one patient. Cutaneous toxicities were in general mild and resolved spontaneously. It has been suggested [22] that dexamethasone prophylaxis may ameliorate these toxicities. Grade 4 neutropenia was noted in two patients but was uncomplicated.

However, two of eight patients at our recommended dose discontinued treatment prematurely because of treatment-related toxicity and six required dose reductions during their planned treatment course.

It should also be noted that in a phase II study of 12 mg/m^2 single-agent OSI-7904L administered first-line in advanced gastric/gastroesophageal adenocarcinoma [22], although 90% of patients were of PS0/1, 12 of 50 needed dose reductions primarily due to cutaneous reactions, oral mucositis and diarrhea. Five further patients withdrawn during cycle 1 would also have required a dose reduction if they had continued on study. This suggests that up to a third of good performance status patients with advanced cancer fail to tolerate 12 mg/m^2 single agent OSI-7904L, supporting our decision not to escalate the OSI-7904L dose in this study further.

Partial radiological disease responses were documented in two patients. Both had received prior TS inhibitors for advanced disease but not irinotecan or oxaliplatin. Both responses occurred at levels below the recommended dose although four of eight patients who were treated at this level had stable disease at completion of protocol therapy. It is notable that in the phase I study of OSI-7904L [10], four out of eleven patients with disease stabilization were treated at doses $\leq$50% of the final recommended dose of 12 mg/m^2. Elevations in plasma 2′-deoxyuridine, a surrogate marker for in vivo TS inhibition [26] were noted in that study at all doses $\geq$3.2 mg/m^2 indicating target effects occurring below recommended dose. This also reinforces our decision not to proceed further with our planned dose escalation.

As previously reported [10, 22], OSI-7904L plasma pharmacokinetics showed significant interpatient variability. Beutel et al. [10] demonstrated that the cause of this variability was differences in the proportion of drug cleared in the α phase and hypothesized that this might be related to differences in liposomal uptake by the reticuloendothelial system or a rapid drug release phase in a proportion of patients. Unfortunately, the development of an assay for free (ultrafilterable) OSI-7904 did not elucidate this as, although free OSI-7904 concentrations were measurable these were 1400-fold lower than total OSI-7904 concentrations and rapidly fell below the limit of quantitation. Within the constraints of our small patient population, we could not detect a significant correlation between OSI-7904 $AUC_{0-\infty}$ and cycle 1 toxicity and pharmacogenomic markers also failed to predict toxicity or efficacy. However, in the larger gastric carcinoma study [22], high plasma AUC for OSI-7904L was significantly associated with the development of grade 3 or 4 toxicity.

In summary, we have identified a recommended dose regimen for the use of OSI-7904L and oxaliplatin in CRC. However, the unpredictable pharmacokinetics of OSI-7904L, and the wide availability and efficacy of oral TS inhibitors mean its further development needs to be considered carefully. This is particularly apposite given the patient resources required to further develop the incorporation of targeted agents into CRC treatment.

Acknowledgments The authors would like to thank Dr. D. W. Drolet for his assistance in the pharmacokinetic analyses.

Funding source The study sponsor was OSI Pharmaceuticals Inc. Study design, data collection, interpretation and decision to submit the manuscript for publication were undertaken by the EORTC in conjunction with the authors.

Conflict of interest statement J. Chick is an employee of OSI Pharmaceutical Inc. and hold share options in this company. None of the other authors have any conflict of interest.

References

1. Colorectal Cancer Collaborative Group (2000) Palliative chemotherapy for advanced colorectal cancer: systematic review and meta-analysis. BMJ 321:531–535
2. Douillard JY, Cunningham D, Roth AD et al (2000) Irinotecan combined with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multi-centre randomized trial. Lancet 355:1041–1047
3. De Gramont A, Figer A, Seymour M et al (2000) Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment for metastatic colorectal cancer. J Clin Oncol 18:2938–2947
4. Tournigand C, Andre T, Achille E et al (2004) FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol 22:229–237
5. Longley DB, Harkin DP, Johnston PG (2003) 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 3:330–338
6. De Gramont A, Bosset JF, Milan C et al (1997) Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous for advanced colorectal cancer: a French intergroup study. J Clin Oncol 15:808–815
7. Van Cutsem E, Twelves C, Cassidy J et al (2001) Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer. J Clin Oncol 19:4097–4106
8. Douillard JY, Hoff PM, Skillings JR et al (2002) Multicenter phase III study of uracil/ tegafur and oral leucovorin versus fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 20:3605–3616
9. Desjardins J, Emerson DL, Colagiovanni DB, Abbott E, Brown EN, Drolet DW (2004) Pharmacokinetics, safety and efficacy of a liposome encapsulated thymidylate synthase inhibitor, OSI-7904L [(S)-2-[5-[1,2-dihydro-3-methyl-1-oxobenzo[*f*]quinazolin-9-yl) methyl]amino-1-oxo-2-isoindolynl]-glutaric acid] in mice. J Pharmacol Exp Ther 309:894–902
10. Beutel G, Glen H, Schöffski P et al (2005) Phase I study of OSI-7904L, a novel liposomal thymidylate synthase inhibitor in patients with refractory solid tumors. Clin Cancer Res 11:5487–5495
11. Winski S, Nilsson C, Brown E et al (2002) In vivo antitumor efficacy of liposomal OSI-7904L in human tumor xenografts. NCI-EORTC-AACR Molecular targets and cancer therapeutics meeting, 2002 Abstract 119
12. Pendyala L, Leichman CG, Clark K et al (2001) Oxaliplatin influences the intra-tumoral expression of γ-glutamylcysteine synthetase and thymidylate synthase genes in patients with esophagus cancer. Proc Am Soc Clin Oncol 20, 351a
13. Kawakami K, Omura K, Kanehira E et al (1999) Polymorphic tandem repeats in the thymidylate synthase gene is associated with its protein expression in human gastrointestinal cancers. Anticancer Res 19:3249–3252
14. Chen J, Hunter DJ, Stampfer MJ et al (2003) Polymorphism in the thymidylate synthase promoter enhancer region modifies risk and survival of colorectal cancer. Cancer Epidemiol Biomarkers Prev 12:958–962
15. Mandola MV, Stoehlmacher J, Muller-Weeks S et al (2003) A novel single nucleotide polymorphism within the 5′ tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Res 63:2898–2904

16. Morganti M, Ciantelli M, Giglioni B et al (2005) Relationships between promoter polymorphisms in the thymidylate synthase gene and mRNA levels in colorectal cancers. Eur J Cancer 41:2176–2183
17. Gurubhagavatula S, Liu G, Park S et al (2004) XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. J Clin Oncol 22:2594–2601
18. Spitz MR, Wu X, Wang Y et al (2001) Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res 61:1354–1357
19. Stoehlmacher J, Park DJ, Zhang W et al (2004) A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. Br J Cancer 91:344–354
20. Cancer Therapy Evaluation Programme (2003) Common Terminology Criteria for Adverse Events, Version 3.0. DCTD, NCI, NIH, DHHS. December 12 2003. (http://www.ctep.cancer.gov/reporting/ctc_v3.0)
21. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205–216
22. Falk SJ, Anthoney A, Eatock M et al (2006) Multicentre phase II pharmacokinetic and pharmacodynamic study of OSI-7904L in previously untreated patients with advanced gastric or gastro-esophageal junction adenocarcinoma. Br J Cancer 95:450–456
23. Graham MA, Lockwood GF, Greenslade D et al (2000) Clinical pharmacokinetics of oxaliplatin: a critical review. Clin Cancer Res 6:1205–1218
24. Extra JM, Espie M, Calvo F et al (1990) Phase I study of oxaliplatin in patients with advanced cancer. Cancer Chemother Pharmacol 25:299–303
25. Maindrault-Goebel F, de Gramont A, Louvet C et al (2001) High-dose intensity oxaliplatin added to the simplified bimonthly leucovorin and 5-fluorouracil regimen as second-line therapy for metastatic colorectal cancer (FOLFOX 7). Eur J Cancer 37:1000–1005
26. Ford HER, Mitchell F, Cunningham D et al (2002) Patterns of elevation of plasma 2′-deoxyuridine, a surrogate marker of thymidylate synthase (TS) inhibition, after administration of two different schedules of 5-fluorouracil and the specific TS inhibitors raltitrexed (Tomudex) and ZD9331. Clin Cancer Res 8:103–109