EUROPEAN JOURNAL OF CANCER 45 (2009) 1935–1949

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

A review on the use of molecular markers of cytotoxic therapy for colorectal cancer, what have we learned?

Miriam Koopman[a,c], *Sabine Venderbosch*[b,c], *Iris D. Nagtegaal*[b], *Johan H. van Krieken*[b], *Cornelis J. Punt*[a,*]

[a]*Department of Medical Oncology, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands*
[b]*Department of Pathology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands*

ARTICLE INFO

Article history:
Received 15 April 2009
Accepted 20 April 2009
Available online 25 May 2009

Keywords:
Colorectal cancer
5FU
Capecitabine
Irinotecan
Oxaliplatin
Molecular markers
DPD
TP
dMMR
TS

ABSTRACT

Background: Over the past decades, significant progress has been achieved in the cytotoxic treatment of colorectal cancer (CRC) by the use of fluoropyrimidines, irinotecan and oxaliplatin. However, as not all patients do respond to chemotherapy, there is a need for predictive and prognostic factors in order to optimise the treatment for individual patients. Although many potential molecular markers have been studied, none of these have been implemented in the standard of care for colorectal cancer patients.

Method: We performed a review of the data on the prognostic and/or predictive value of molecular markers for cytotoxic drugs in CRC. The following markers were included: dihydropyrimidine dehydrogenase, orotate phosphoribosyl transferase, thymidine phosphorylase, thymidylate synthase, mismatch repair deficiency, topoisomerase 1, excision cross-complementing gene and carboxylesterases.

Results: With the exception of mismatch repair deficiency, these molecular markers showed divergent and inconsistent results on their prognostic and/or predictive value. This underscores the complexity of the role of these markers.

Conclusions: We conclude that further retrospective testing of these markers is unlikely to add clinically useful results. More definite results may only be expected when these markers are included in the design of prospective randomised studies.

1. Introduction

Over the past decades, significant progress has been achieved in the treatment of colorectal cancer (CRC) by advances in surgery, radiotherapy and systemic therapy. The most important prognostic factor in CRC is the stage at diagnosis as defined by the American Joint Committee on Cancer (AJCC). With increasing stages I to IV the 5-year overall survival declines from greater than 90% to less than 10%.[1]

Surgery with curative intent is the treatment of choice in stages I–III colon cancer. Adjuvant chemotherapy significantly improves the overall- and disease-free survival in stage III and possibly high-risk stage II patients.[2] Initially, adjuvant therapy consisted of 5-fluorouracil (5FU), 5FU plus levamisole, or 5FU plus leucovorin.[2–4] This benefit can also be achieved by capecitabine monotherapy.[5] The combination of a fluoropyrimidine with oxaliplatin further increased the three-year disease-free survival with approximately 6%.[6,7] The survival benefit of

* *Corresponding author:* Tel.: +31 24 3610353; fax: +31 24 3540788.
E-mail address: c.punt@onco.umcn.nl (C.J. Punt).
[c] Miriam Koopman and Sabine Venderbosch equally contributed to this manuscript.
0959-8049/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2009.04.023

adjuvant treatment in the overall population with stage II disease is generally not considered as clinically relevant.[6,8]

In resectable rectal cancer, neoadjuvant radiotherapy with or without chemotherapy followed by TME surgery is the treatment of choice.[9] The value of adjuvant chemotherapy is still a subject of debate.[10,11]

For patients with distant irresectable metastatic CRC there are no curative treatment options and 5FU has been the standard care for decades, resulting in a survival benefit of more than 6 months.[12,13] Oral fluoropyrimidines like capecitabine and UFT resulted in a comparable efficacy with less toxicity compared to intravenous 5FU/LV.[14,15] Oxaliplatin and irinotecan have become available for first and second-line treatment of advanced CRC, which further extended the overall survival (OS) to approximately 14–20 months.[16,17] There is no optimal sequence for the use of irinotecan and oxaliplatin in terms of efficacy, a preference may be based on the difference in toxicity pattern between these cytotoxics.[18] A retrospective analysis showed that the OS is increased when all three effective cytotoxic drugs are made available to patients.[19] Two recently published studies prospectively investigated the use of sequential versus combination chemotherapy with the use of a fluoropyrimidine, irinotecan and oxaliplatin.[20,21] Both studies showed no OS benefit for combination chemotherapy as compared to sequential chemotherapy.

The development of new targeted drugs, such as vascular endothelial growth factor (VEGF)- and epidermal growth factor receptor (EGFR)-antibodies, have added further clinical benefit to patients with metastatic CRC.[12,22,23] However, cytotoxic chemotherapy remains the backbone of treatment.

Two important conclusions can be drawn from the published results on chemotherapy in metastatic CRC. First, there is no single regimen that is clearly superior. This implies that several options are available which should be discussed between doctor and patient. Second, not all patients will benefit from treatment. This implies that many patients are unnecessarily exposed to the toxic and sometimes lethal effects of chemotherapy.

Although clinical characteristics may be used to select patients for treatment, there is an urgent need for the development of prognostic and predictive markers. Biomarkers correlating with outcome are categorised as either prognostic or predictive markers.[24] Prognostic biomarkers correlate with outcome independent of treatment, and predictive markers correlate with the impact of specific treatment on outcome. With the increased knowledge on the molecular pathways by which cytotoxic drugs exert their effects, it became possible to study the role of various key enzymes and targets involved.

We performed a review of the published data on the prognostic and predictive value of molecular markers for cytotoxic drugs that are used in the standard treatment of CRC. For a better understanding, a short overview of the metabolism and mechanisms of action of these drugs is also presented.

2. Methods

We reviewed the literature on the prognostic and/or predictive value of molecular markers in relation to outcome on standard cytotoxic chemotherapy of patients with CRC in all stages of disease. The primary outcomes of interest were OS, disease-free survival (DFS), progression-free survival (PFS), and response rate (RR).

A search was conducted of Medline from January 1980 to July 2008 with an English language restriction. The search strategy included the following keywords (MESH terms) in different combinations: colorectal cancer, biomarkers, predictive, prognostic, outcome, survival, response fluoropyrimidines, irinotecan, oxaliplatin, immunohistochemistry (IHC), RNA analysis, protein expression and paraffin embedded material. Individual markers were also included in searches. Only clinical studies were included, and in case of duplicate publications the latest and most complete study was included. Studies published in abstract form only or articles published in non-peer reviewed journals were excluded. Because of the widely disparate end-points and methods of the studies, formal meta-analytic techniques could not be used. We will discuss the following chemotherapeutic drugs: 5-fluorouracil (5FU), capecitabine, irinotecan, oxaliplatin, and the following biomarkers: dihydropyrimidine dehydrogenase (DPD), orotate phosphoribosyl transferase (OPRT) thymidine phosphorylase (TP), thymidylate synthase (TS), mismatch repair deficiency (dMMR), topoisomerase 1 (topo-I), excision cross-complementing gene (ERCC1) and carboxylesterases (CES).

3. Fluoropyrimidines

3.1. 5-Fluorouracil (5FU)

5FU belongs to the class of antimetabolite drugs and is administered intravenously. 5FU is an analogue of uracil using the same facilitated transport mechanism for entering the cell.[25,26] Uracil, which is incorporated into RNA and methylated to generate thymidine for DNA production, is preferentially used by cancer cells and a fluorinated analogue of this base might selectively alter cancer cell metabolism. The pathway of 5FU metabolism is shown in Fig. 1.[26–35]

An alternative route of metabolism of 5FU is mediated by the inhibition of thymidylate synthase (TS). Thymidylate can be salvaged from thymidine through the action of thymidine kinase (TK), thereby alleviating the effects of TS deficiency. This salvage pathway represents a potential mechanism of resistance to 5FU.[26,36] More than 80% of the administered 5FU is primarily catabolised in the liver, in which organ the enzyme dihydropyrimidine dehydrogenase (DPD) is expressed.[30,37] DPD mediates 5FU catabolism, which results in the formation of inactive dihydrofluorouracil (DHFU) that is further degraded to fluoroureidopropionate (FUPA) and the inactive fluoro-β-alanine (F-BAL), which is excreted in urine. DPD is the rate-limiting enzyme in the 5FU catabolism.[26]

3.2. Capecitabine

Capecitabine is an oral fluoropyrimidine, and is absorbed through the intestine as a prodrug.[27] Capecitabine is converted to 5FU by a three-step enzymatic process (Fig. 2).[26] Conversion to 5'-deoxy-5-fluorocytidine (5'-DFCR) by carboxyl-

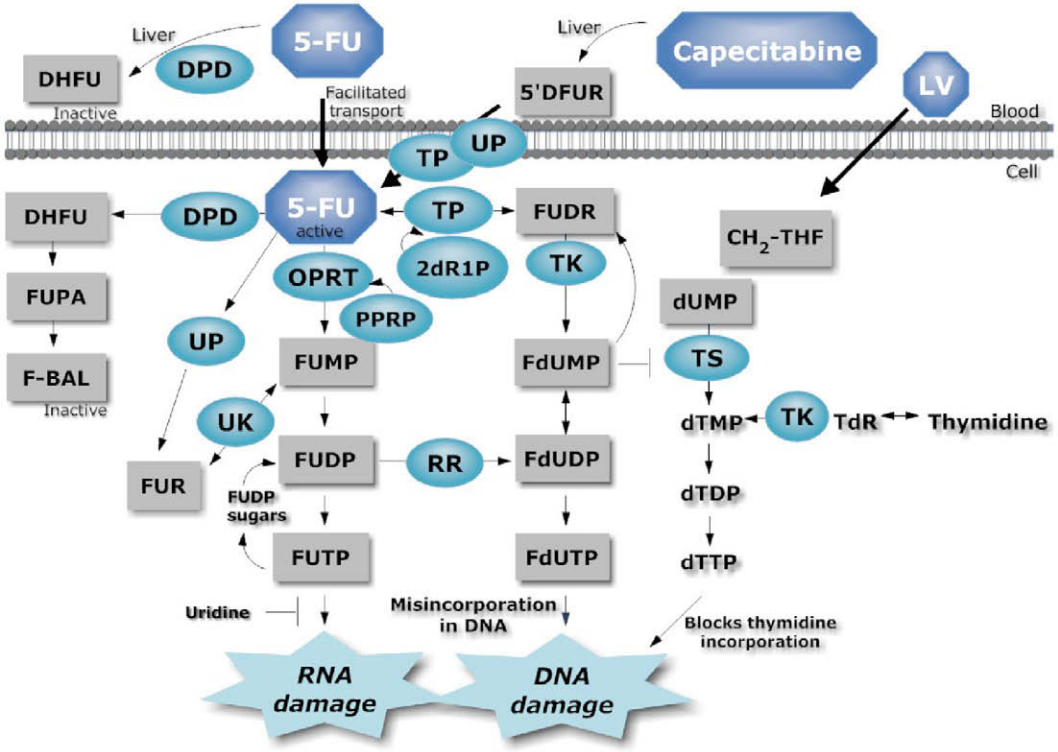

Fig. 1 – The metabolism of fluoropyrimidines (26–35).

esterases in the liver is followed by conversion to 5'-DFUR by cytidine deaminase which is highly expressed in the liver.[38] Subsequently thymidine phosphorylase (TP) and/or uridine phosphorylase (UP) enables the conversion to the active 5FU compound.[39] This final step occurs in tumour as well as in normal tissue, although TP expression is higher in tumour tissue as compared to normal tissue. In terms of efficacy, this gives capecitabine at least a theoretical advantage compared

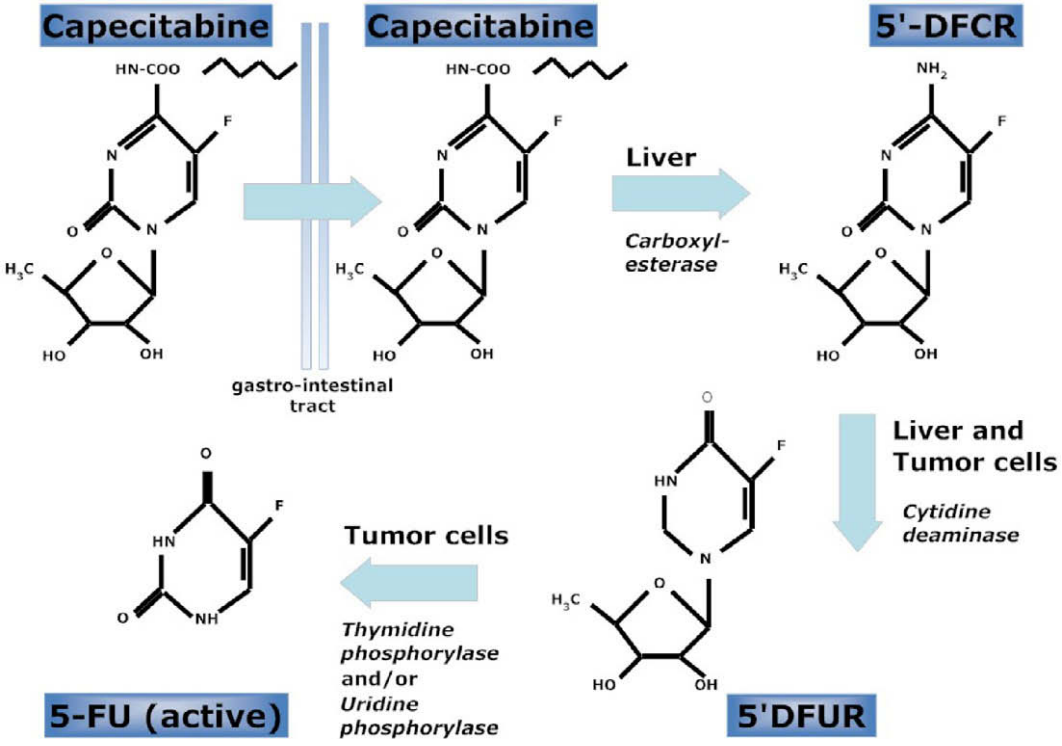

Fig. 2 – The metabolism of capecitabine.[26,27,156–160]

to 5FU.[40–43] After transformation of capecitabine to the active 5FU compound, metabolism will further proceed along the 5FU pathway (Fig. 1).

4. Molecular markers and outcome of treatment on fluoropyrimidines

The expression of several key enzymes in the 5FU pathway has been studied to investigate a possible correlation with the outcome of treatment. The result of our search on fluoropyrimidines and biomarkers is presented in Table 1. The content of this table is derived from a search in which clinical studies in CRC patients were selected in which the expression of DPD and/or OPRT and/or TP and/or TS was examined by immunohistochemistry (IHC) and/or RT-PCR analysis on paraffin embedded material of the primary tumour and/or metastases.

4.1. *Dihydropyrimidine dehydrogenase (DPD)*

Low DPD expression theoretically leads to a decreased catabolism of 5FU and thus to a more effective intracellular 5FU concentration. *In vitro*, DPD activity in tumour cells has indeed shown to be an independent factor related to 5FU sensitivity.[44] *In vivo*, several studies in the adjuvant[45–51] or metastatic[52,53] setting have investigated the relation between DPD expression and the outcome of 5FU-based treatment (Table 1). These studies used different methods, patient populations and techniques. Takenoue et al.[54] compared different techniques of determining DPD expression (IHC and mRNA) with the DPD protein level measured by western blot, and found that IHC expression correlated better with protein level than mRNA expression. Studies on DPD expression in primary tumours as determined by IHC and/or RT-PCR with the use of fluoropyrimidines in the adjuvant setting found a significant correlation between a low DPD expression and prolonged overall survival,[45,55] and disease-free survival,[45,48,49] whilst others found only a trend[50] or no correlation at all.[51] In the metastatic setting six studies with small number of patients[52,53,55–58] and only one larger study[59] were performed with conflicting results. In this largest study on capecitabine with or without irinotecan to date we found a predictive value of DPD expression.

4.2. *Orotate phosphoribosyl transferase (OPRT) and uridine phosphorylase (UP)*

OPRT is responsible for the conversion of 5FU to the active metabolite fluorouridine monophosphate (FUMP). A high OPRT expression could therefore, be a predictive factor for response to 5FU-based chemotherapy. Thus far, this has been tested in only three studies (Table 1). Tokunaga et al.[55] found that a high OPRT expression (IHC) in stages II-IV disease was correlated with a better overall survival, which was not observed in a smaller study using RT-PCR.[53] A prognostic value of OPRT expression in both tumour and stromal cells but each with an opposite effect on outcome was an unexpected finding in a retrospective analysis of a large phase III trial.[59]

The low interest for OPRT as a prognostic marker may be explained by the fact that the route of uridine phosphorylase (UP), which bypasses OPRT, has been suggested as the most important step in the fluoropyrimidine pathway (Fig. 1). This may be especially true for capecitabine, since UP may contribute to the conversion of capecitabine to active 5FU (Figs. 1 and 2).[39,60] In colon-cancer cell lines UP was predictive for the cytotoxicity of 5FU[61]; however, *in vivo* there are no data on the correlation between UP expression and the outcome of treatment.

4.3. *Thymidine phosphorylase (TP)*

One of the first steps in the capecitabine pathway is the conversion of 5'-DFUR to the active compound 5FU (Fig. 2). TP and possibly UP are the only known enzymes that constitute the rate-limiting step in this conversion. In addition, TP is involved in the conversion of 5FU to FdUMP, which finally leads to DNA damage (Fig. 1). Therefore, a high TP expression may predict a good response to fluoropyrimidine treatment.

TP is also known as platelet-derived endothelial cell growth factor (PD-ECGF), and this enzyme is believed to have angiogenic properties. The precise mechanisms through which it promotes neoangiogenesis are still not fully elucidated.[62] Neovascularisation is necessary for tumour growth and metastases,[63] and a positive correlation between TP expression and microvessel density (MVD) has been reported.[40,64–67] This suggests that a high TP expression may rather predict a poor outcome.

This dual role of TP may underlie the contradictory results of studies examining the relationship between TP expression and clinical outcome in patients treated with fluoropyrimidines.[29,45,52,53,59,62] The two studies in which capecitabine was used also showed contradictory results.[52,59]

Additional complications in interpreting the results of TP expression involve the use of different techniques as well as its measurement at different sites. Although TP expression appeared comparable in tissue from the primary tumour versus metastases, its association with clinical activity was most pronounced in metastases. Furthermore the predictive value for response to treatment of IHC TP expression was better than for TP gene expression by RT-PCR.[52] TP is expressed at higher levels in tumour cells and in tumour infiltrating stromal cells compared with normal tissue.[27] This suggests a specific role of stroma in the production of angiogenic factors such as TP.[29,68]

4.4. *Thymidylate synthase (TS)*

TS is the rate-limiting enzyme in the '*de novo*' synthesis of 2'deoxy-thymidine-5'-monophosphate (dTMP), which is required for DNA synthesis and repair (Fig. 1). Therefore, TS is the primary target of fluoropyrimidines.

Low levels of TS may lead to more DNA damage. However, as tumour cells are more proliferative compared to normal cells and proliferation requires DNA synthesis, a low TS expression may lead to less synthesis of dUMP and thus to less DNA synthesis. These low TS-containing tumours are less proliferative and may therefore, be associated with a more favourable prognosis. Therefore, a low TS expression may rather be a prognostic than a predictive factor of outcome of fluoropyrimidines.[69]

In vitro, TS is a determinant of the sensitivity to fluoropyrimidines.[70,71] Studies which have focussed on the role of TS

Table 1 – Studies in which the expression of DPD, TP, TS and OPRT was correlated to the outcome of 5FU-based chemotherapy.

Author	Date of publication	Treatment setting	Lesion tested	Method	Number of patients	Longer overall survival				Longer disease-free or progression-free survival				Better response to 5FU-based chemotherapy			
						DPD	TP	TS	OPRT	DPD	TP	TS	OPRT	DPD	TP	TS	OPRT
Leichman et al.[161]	1997	Advanced	Metastasis	RT-PCR	42			Low								Low	
Lenz et al.[162]	1998	Advanced	NS	RT-PCR	36			Low								Low	
Aschele et al.[163]	1999	Advanced	Metastasis	IHC	48			Low				Low				Low	
Triest van et al.[29]	2000	Adjuvant[h]	Primary	IHC	32		~	~			Low	~					
Shirota et al.[a145]	2001	Advanced	Metastasis	RT-PCR	50			Low								Low	
Edler et al.[164]	2002	Adjuvant[h]	Primary	IHC	862			Low				Low					
Allegra et al.[165]	2002	Adjuvant[h]	Primary	IHC	465			~				~					
Allegra et al.[69]	2003	Adjuvant[h]	Primary	IHC	691			Low				Low					
Johnston et al.[166]	2003	Advanced	Primary	IHC	219			~								~	
Paradiso et al.[b120]	2004	Advanced	Primary	IHC	108			~								Low	
Oi et al.[49]	2004	Adjuvant[sf]	Primary	IHC	64					Low							
Westra et al.[51]	2005	Adjuvant[h]	Primary	IHC	391					~		~					
Bendardaf et al.[b167]	2005	Advanced	Primary	IHC	86			Low								Low	
Tokunaga et al.[56]	2005	Advanced	Primary	IHC	150	Low	Low										
Meropol et al.[c52]	2006	Advanced	Primary & metastasis	RT-PCR & IHC	67		High				High			~	High	~	
Ciaparrone et al.[45]	2006	Adjuvant[sf]	Primary	IHC	62	Low	~	Low		Low	~	Low					
Lassmann et al.[48]	2006	Adjuvant[h]	Primary	RT-PCR	102					Low	~	~					
Popat et al.[168]	2006	Adjuvant[h]	Primary	IHC	967			~									
Vallböhmer et al.[b122]	2006	Advanced	Primary	RT-PCR	54	~		~						~		~	
Yanagisawa et al.[b53]	2007	Advanced	Primary	RT-PCR	21	Low	~	~	~					~	~	Low	~
Tokunaga et al.[55]	2007	Advanced/ adjuvant	Primary	IHC	150	Low			High								
Vallböhmer et al.[d58]	2007	Advanced	Primary	RT-PCR	37					Low	~	~		Low	~	~	
Soong et al.[50]	2008	Adjuvant	Primary	IHC	945	~	~	~									
Koopman et al.[e59]	2009	Advanced	Primary	IHC	556	Low	~	~	Low	Low	~	~	Low				

Note: RT-PCR, reverse transcriptase polymerase chain reaction; IHC, immunohistochemical analysis; DPD, dihydropyrimidine dehydrogenase; OPRT, orotate phosphoribosyl transferase; TP, thymidine phosphorylase; TS, thymidylate synthase; ~, not significant.

a based on 5-FU and oxaliplatin as second-line therapy.
b based on 5-FU and irinotecan as first-line therapy.
c based on capecitabine plus irinotecan as first-line therapy.
d based on capecitabine as first-line therapy.
e based on capecitabine versus capecitabine plus irinotecan as first-line therapy.
sf surgery and post-operative fluorouracil.
h heterogenous group of therapies given.

expression *in vivo* have used different detection methods (IHC, mRNA), and material (metastases or primary tumours). The results of these studies are therefore, difficult to compare, as was already concluded in the meta-analysis of Popat et al.[72] In this analysis patients with tumours expressing high levels of TS appeared to have a worse overall survival compared to patients with tumours expressing low levels. However, the heterogeneity of the studies and a possible publication bias do not allow a straightforward conclusion. Conflicting results have also been published on TS expression in metastases versus the primary tumour.[52,73,74] Therefore, additional studies with consistent methodology are needed to define the precise prognostic value of TS.[72] However, a prognostic value of TS was not observed in one of the largest retrospective analyses to date,[59] and therefore, it may be questioned whether further retrospective testing of this marker will provide useful data.

4.5. *Mismatch repair deficiency (dMMR)*

One of the first indications that molecular characteristics might impact the results of treatment came from patients with a mismatch repair deficiency (dMMR).[75–79] The MMR system is responsible for reparation of insertions and deletions in microsatellite regions of DNA. dMMR is probably involved in fluoropyrimidine resistance. dMMR allows tumour cells to tolerate DNA damage and replicate, instead of undergoing cell cycle arrest or death.[80]

dMMR is relatively common in CRC, as part of a hereditary non-polyposis CRC syndrome (Lynch syndrome 0.8–5%), as well as a sporadic finding in 10–20% of patients, which is largely due to *MLH1*-promoter hypermethylation.[81–85] Patients with dMMR tumours have a better prognosis compared to patients with an intact MMR system.[77,78,86–88] Several studies investigated if chemosensitivity is implied in the better prognosis of patients with a dMMR tumour. *In vitro* studies have shown dMMR cell lines to be resistant to 5FU,[89,90] but not to oxaliplatin[91,92] or irinotecan.[93] *In vivo*, most studies have been performed in the adjuvant setting, probably because the incidence of dMMR in metastatic patients is low.[94] Retrospective studies in stages II–III CRC patients reported conflicting results on the correlation between dMMR and outcome on treatment.[75,76,79,95] However, in most of these studies a low sensitivity of dMMR tumours to 5FU was observed, which was confirmed by a recent pooled reanalysis of randomised chemotherapy trials.[96] The ASCO 2006 and European 2007 guidelines do not recommend the use of dMMR as a prognostic and/or predictive marker in this setting,[97,98] but most likely this will happen in the near future. Furthermore, dMMR as a predictive marker is feasible, as dMMR detected by IHC for MLH1 and MSH2 has been shown to provide a rapid, cost-effective, sensitive (92.3%) and specific (100%) method for screening for DNA MMR defects in colorectal tumours.[99]

5. Irinotecan

Irinotecan is a topoisomerase 1 (topo-I) inhibitor. It acts as a prodrug of SN-38 (7-ethyl-10-hydroxycamptothecin), which is 100- to 1000-fold more cytotoxic than the parent drug,[100] and is most cytotoxic to cells in the S-phase (Fig. 3).[101] Irinotecan associates with the DNA-topo-I complex, and upon stabilization single stranded breaks are induced. Because of the high reversibility the single strand DNA breaks alone do not result in cell death. Irreversible DNA damage occurs when DNA synthesis is ongoing and the replication fork enters a cleavable complex, resulting in double stranded breaks and ultimately cell death.[102]

Irinotecan is metabolized in blood to the active metabolite SN-38 by butyrylcholinesterases.[103] Irinotecan and SN-38 are present in two distinguishable forms with a pH-dependent equilibrium: an active α-hydroxy-δ-lactone ring and an inactive carboxylate structure. In blood, irinotecan is predominantly present in the inactive carboxylate form, whilst SN-38 exists predominantly in the active lactone form.[104,105] The lactone forms of irinotecan and SN-38 are taken up by intestinal epithelial cells and colon carcinoma cells by passive diffusion. The carboxylate form is absorbed by an active pH-dependent transport mechanism.[106]

In blood, 80% of irinotecan is bound to erythrocytes, whereas SN-38 is almost completely bound to albumin and lymphocytes.[107,108] In the presence of albumin, the lactone forms of irinotecan and SN-38 are more stable and available in higher amounts.[109]

SN-38 is transported from the blood to the liver by the organic anion-transporting polypeptide (OATP1B1),[110] with preliminary evidence for the bidirectional transport of OATP1B1 *in vitro*. Irinotecan is converted in the liver to SN-38 by two carboxylesterases, human carboxylesterase-1 (CES1) and CES2, of which the latter is the most relevant enzyme in this process.[111,112] Carboxylesterases are categorised as phase-I drug-metabolizing enzymes, which are able to hydrolyse a variety of ester-containing drugs and prodrugs.[113]

The conversion of SN-38 to inactive SN-38 glucuronide (SN-38G) is mediated by the uridine diphosphoglucuronosyltransferase (UGT) family, mainly by UGT1A1, UGT1A6 and UGT1A9.[114,115] Irinotecan can also be converted to the inactive forms APC and NPC, which is mediated by cytochrome P-450 isoform 3A (CYP3A4) and CYP3A5, although the latter enzyme shows only weak catalytic activity.[116] Interestingly, CES1 and CES2 are able to convert the inactive NPC metabolite to active SN-38 by hydrolysis.[104,117]

Irinotecan, SN-38 and SN-38G are transported from bile to the intestine by transporters belonging to the ATP-Binding Cassette (ABC) genes which are expressed in a wide variety of normal tissues. The ABC transporters play a role in the development of multi-drug resistance by cancer cells, of which the ABCB1, ABCC2, and ABCG2 genes are involved in the transport of irinotecan, SN-38G, and SN-38.[118]

6. Molecular markers for the outcome of treatment of irinotecan

6.1. *Topoisomerase I (topo-I)*

Topo-I plays an essential role in DNA replication by relaxing the super-coiled helix with single-stranded DNA breaks. The cytotoxic effect of irinotecan is based on inhibiting topo-I by stabilizing the DNA-topo-I complex, leading to replication arrest and apoptosis. Topo-I is overexpressed in CRC in

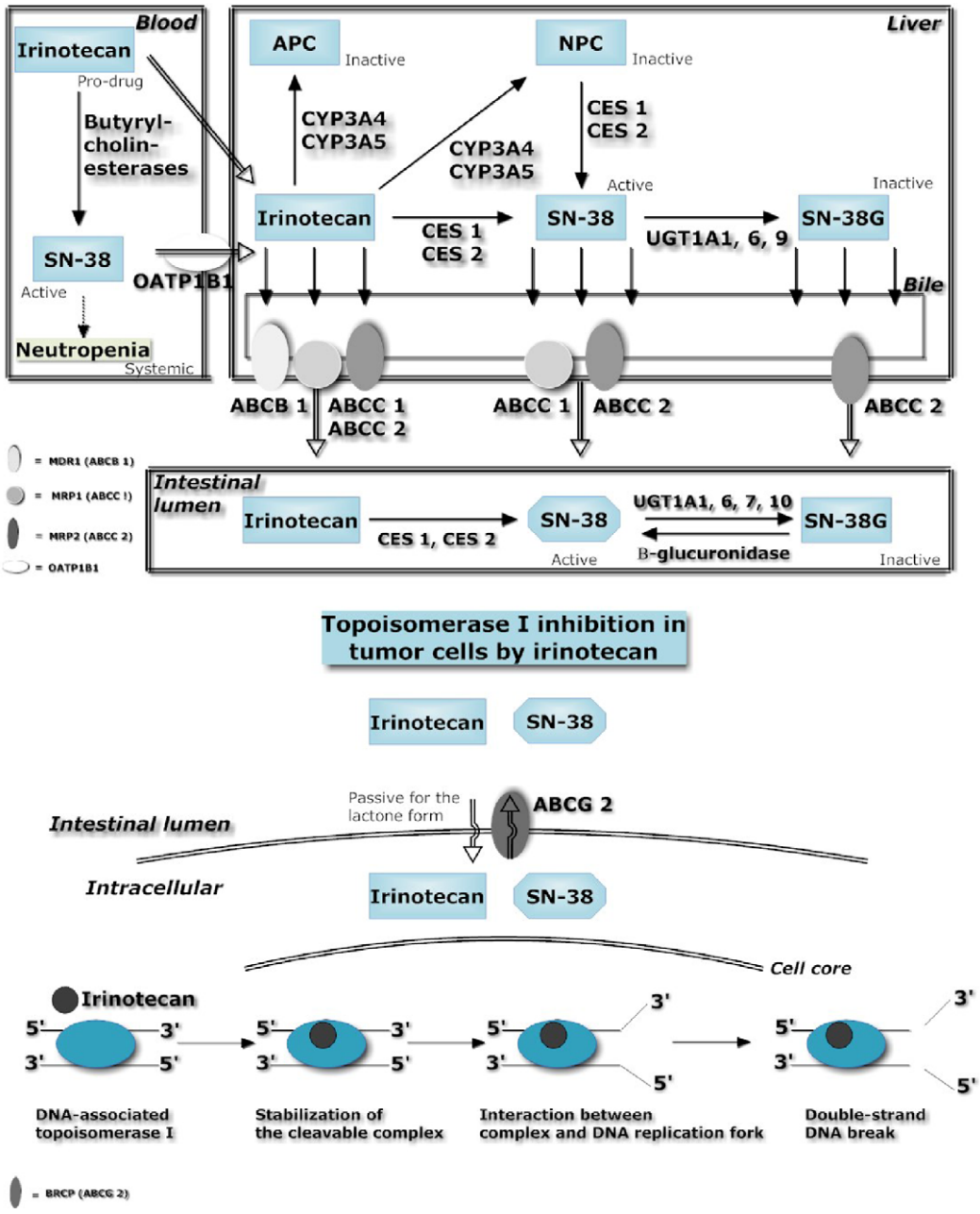

Fig. 3 – The metabolism of irinotecan.[107,116,117,169–173]

43–51%.[119,120] In cell lines a positive correlation between irinotecan sensitivity and topo-I activity was found.[121] This was not confirmed in small non-randomised clinical studies.[120,122] However, in a recent large randomised study in patients with advanced CRC, topo-I expression was identified as both a predictive marker associated with the benefit of either irinotecan or oxaliplatin, and a prognostic marker associated with outcomes with 5FU therapy alone.[123] Further studies are needed to establish the definite role of topo-I expression and the outcome on irinotecan in CRC.

6.2. *Carboxylesterases (CES1 and CES2)*

Carboxylesterases (CES) are involved in the hydrolysis of irinotecan (Fig. 3). CES1 and CES2 are expressed not only in normal liver tissue but also in colon-cancer cells, and may therefore, have a role in the conversion of irinotecan to SN-38 in tumour cells.[124–126] A high expression of CES in tumour cells may be correlated with a better response to irinotecan chemotherapy. To date, no data on the correlation of the expression of CES in tumour tissue in relation to the response to irinotecan are available.

7. Oxaliplatin

Oxaliplatin (trans-l-diaminocyclohexane oxalatoplatinum) is a third-generation platinum compound, which was selected for further investigations based on its water solubility (about eightfold more soluble than cisplatin), less toxic side-effects, promising antitumour activity on the L1210 cell line, and lack

of cross-resistance with cisplatin.[127,128] Oxaliplatin showed *in vitro* and *in vivo* antitumour activities in CRC, where other platinum compounds, cisplatin and carboplatin, failed to show any activity.[80] Moreover, oxaliplatin proved to be synergistic with other anticancer agents, including 5FU and irinotecan.[129]

The metabolism of oxaliplatin is shown in Fig. 4. The oxaliplatin molecular structure consists of a central platinum atom (Pt), surrounded by a 1,2-diaminocyclohexane group (DACH) and a bidentate oxalate ligand.[130] Oxaliplatin binds irreversibly to erythrocytes and/or forms complexes with albumin and other plasma proteins. The remaining free fraction of oxaliplatin is biotransformed non-enzymatically and subsequently forms complexes with chloride, glutathione, methionine and cysteine.[131–133] The main mechanism of action is mediated through the formation of DNA adducts,

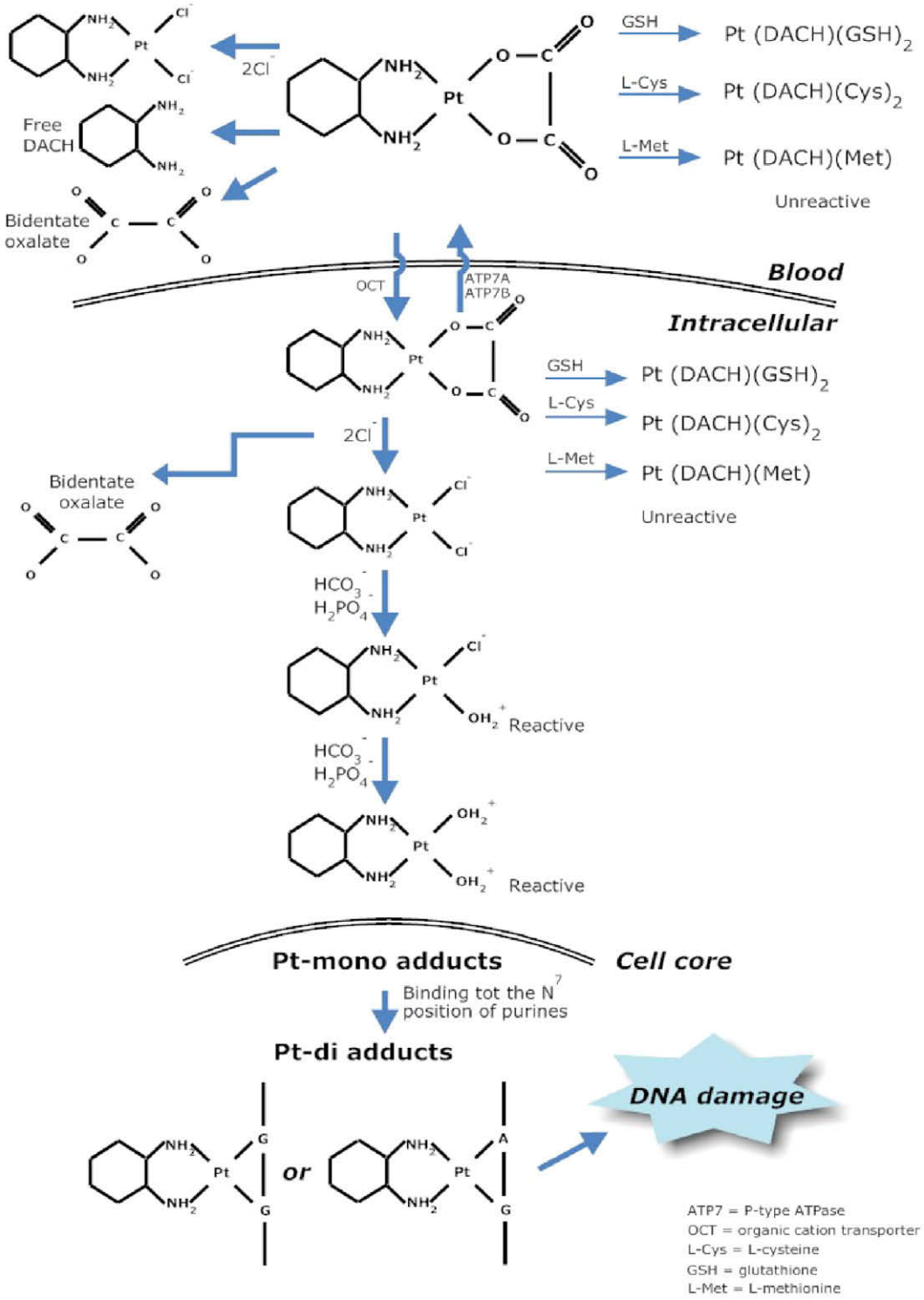

Fig. 4 – The metabolism of oxaliplatin.[128,134,174–179]

which is thought to be related to the anti-tumour effects of oxaliplatin.[130,134] An important factor is the induction of apoptosis by the primary DNA-Pt lesions, which is possibly enhanced by a contribution of targets other than DNA.[135]

Cellular uptake and efflux determine the concentration of oxaliplatin in the intracellular fluid. In plasma, extracellular conjugation of oxaliplatin to plasma proteins (mainly albumin) results in renal excretion of inactive drug species. Once inside the cell the oxaliplatin prodrug is activated by the conversion to monochloro, dichloro and diaquo compounds by non-enzymatic hydrolysis and displacement of the oxalate group, which leads to the formation of DNA adducts. The kinetics of hydrolysis differs amongst platinum compounds, being slower for oxaliplatin than for cisplatin. Intracellular conjugation to glutathione effectively inactivates these highly reactive oxaliplatin species before DNA damage occurs, which is followed by cellular excretion into plasma. DNA damage is repaired by nucleotide excision repair (NER), base excision repair (BER), and replicative bypass.[130] Sensitivity to oxaliplatin can be induced by five different pathways: a decrease in cellular uptake, an increase in cellular efflux, inactivation of the cytotoxic form of drug by L-methionine, L-cysteine and glutathione (GSH), inactivation of the monoadducts by L-methionine, L-cysteine and glutathione (GSH) and an increase of the nuclear excision repair (NER) pathway.[128,136] Only recently several influx and efflux transporters like organic cation transporters (OCT) 1, 2 and 3 (SLC22A1, SLC22A2 and SLC22A3),[137,138] copper efflux transporters, P-type ATPases, ATP7A and ATP7B[139,140] have been identified, which may play an important role in determining tumour sensitivity and/or resistance to anticancer agents. The mismatch repair complex seems not to be involved in resistance to oxaliplatin, although it is an important resistance mechanism to other platinum drugs.[91]

8. Molecular markers for the outcome of treatment of oxaliplatin

8.1. *Excision cross-complementing gene (ERCC1)*

ERCC1 is an excision nuclease within the NER pathway which plays a major role in repairing platinum-induced DNA adducts. ERCC1 forms a heterodimer with xeroderma pigmentosum group F (XPF), which stabilizes this endonuclease. As a unit, they execute the 5' incision into the DNA strand relative to the site of DNA damage in the NER process, thereby removing the modified nucleotides.[141] This DNA repair is involved in the sensitivity/resistance to platinum-based chemotherapy *in vitro*[142] and *in vivo*.[143,144] Based on the pathway of oxaliplatin a low ERCC1 expression may lead to a decrease in DNA repair, which is a positive feature for the induced apoptosis. ERCC1 expression as a predictive factor for response has been investigated in only a small number of studies. A low ERCC1 gene expression was associated with a better overall survival in advanced CRC patients treated with oxaliplatin based chemotherapy.[145] In a phase III trial ERCC1 protein expression was investigated in 506 patients, and was not prognostic for outcome in patients treated with capecitabine plus oxaliplatin in third- and second-line treatment.[59] Others found an association between a high ERCC1 expression and a good response to irinotecan-based chemotherapy.[122] This was explained as that a high ERCC1 expression represented increased DNA repair which would make these cells more vulnerable to topo-I inhibitors. Taken together, the role of ERCC1 as a predictive factor for the response to chemotherapy is still uncertain.

9. Conclusions

We reviewed the potentially prognostic and/or predictive markers of currently used cytotoxic drugs in the treatment of CRC. These markers were selected based on the molecular mechanism of action of these drugs. Only one marker, dMMR, was recently confirmed as relevant in clinical decision making.[96] For all other markers negative or, at best, inconsistent results have been published, as shown in this review. Several comments can be made on this disappointing outcome.

It should be noted that amongst studies there is no consistent use of the definitions of the prognostic role versus predictive role of biomarkers,[24] which hampers cross-study comparisons.

Furthermore, the majority of the published studies concern a retrospective analysis of data which are derived from mostly non-randomised and relatively small-sized and therefore, underpowered studies. The comparison made between different studies is further hampered by the fact that different techniques have been used to examine the expression of biomarkers, such as protein levels, IHC and RT-PCR. Studies comparing these techniques have revealed conflicting results.[52,54,146,147] Also, since CRC may arise through different molecular pathways, it is questionable whether a single biomarker may play a relevant role in all patients with this disease. Analysis of proteins in tumour tissue is hampered by the fact that there is only a limited standardisation of preanalytical procedures and that the most commonly used methods are semi-quantitative at best.

Another possible confounding factor is that in most biomarker studies the primary tumour was used as the source of investigation. However, conflicting results have been published on the expression of these biomarkers in primary tumour versus distant metastases.[52,73,148,149] Therefore, the results of studies with advanced CRC patients should be interpreted with caution. It may also be questioned whether the testing of biomarkers should be limited to tumour tissue given the increasing awareness that surrounding stromal tissue and tumour infiltrating stromal cells play an important role in angiogenesis and immune response and therefore, prognosis.[150–152] Our own findings on the role of OPRT stress the importance of the role of stroma in CRC.[59]

Finally, Kyzas et al.[153] have noted that the majority of studies on predictive/prognostic molecular markers highlight statistically significant results. In the rare articles where no prognostic markers are presented as significant, the authors often have other (non-prognostic) statistically significant analyses to show, they expand on the importance of non-significant trends, or defend the importance of the cancer marker with other arguments. Eventually, totally 'negative' articles on prognostic cancer markers represent less than 1.5% of this literature that is served by a wide variety of jour-

nals. Under strong reporting bias, statistical significance loses its discriminating ability for the importance of prognostic markers.

What have we learned from all these studies? The often divergent and inconsistent results of the studies presented to date underscore the complexity of these biomarkers. International guidelines for the design of biomarker studies and the validation of their results should be developed.[24,154,155] We conclude that further retrospective testing of predictive molecular markers of cytotoxic treatment is unlikely to provide clinically relevant and useful results. This may only be expected when these markers are tested in a prospective manner. Such studies will; however, be more difficult to perform due to their complex design.

Conflict of interest statement

None declared.

REFERENCES

1. O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American joint committee on cancer sixth edition staging. *J Natl Cancer Inst* 2004;**96**:1420–5.
2. Gill S, Loprinzi CL, Sargent DJ, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol* 2004;**22**:1797–806.
3. Buyse M, Zeleniuch-Jacquotte A, Chalmers TC. Adjuvant therapy of colorectal cancer. Why we still don't know. *JAMA* 1988;**259**:3571–8.
4. Moertel CG, Fleming TR, Macdonald JS, et al. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. *Ann Intern Med* 1995;**122**:321–6.
5. Twelves C, Wong A, Nowacki MP, et al. Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* 2005;**352**:2696–704.
6. Andre T, Boni C, Mounedji-Boudiaf L, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004;**350**:2343–51.
7. Kuebler JP, Wieand HS, O'Connell MJ, et al. Oxaliplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: results from NSABP C-07. *J Clin Oncol* 2007;**25**:2198–204.
8. Quasar Collaborative Group, Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet* 2007;**370**:2020–9.
9. Kapiteijn E, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. *N Engl J Med* 2001;**345**:638–46.
10. Collette L, Bosset JF, den Dulk M, et al. Patients with curative resection of cT3–4 rectal cancer after preoperative radiotherapy or radiochemotherapy: does anybody benefit from adjuvant fluorouracil-based chemotherapy? A trial of the European organisation for research and treatment of cancer radiation oncology group. *J Clin Oncol* 2007;**25**:4379–86.
11. Bosset JF, Collette L, Calais G, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 2006;**355**:1114–23.
12. Golfinopoulos V, Salanti G, Pavlidis N, Ioannidis JP. Survival and disease-progression benefits with treatment regimens for advanced colorectal cancer: a meta-analysis. *Lancet Oncol* 2007;**8**:898–911.
13. Simmonds PC. Palliative chemotherapy for advanced colorectal cancer: systematic review and meta-analysis. Colorectal cancer collaborative group. *BMJ* 2000;**321**:531–5.
14. Hoff PM, Ansari R, Batist G, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. *J Clin Oncol* 2001;**19**:2282–92.
15. van Cutsem E, Twelves C, Cassidy J, et al. Oral capecitabine compared with intravenous fluorouracil plus leucovorin in patients with metastatic colorectal cancer: results of a large phase III study. *J Clin Oncol* 2001;**19**:4097–106.
16. Cunningham D, Pyrhonen S, James RD, et al. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet* 1998;**352**:1413–8.
17. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000;**18**:2938–47.
18. Punt CJ. Irinotecan or oxaliplatin for first-line treatment of advanced colorectal cancer? *Ann Oncol* 2005;**16**:845–6.
19. Grothey A, Sargent D, Goldberg RM, Schmoll HJ. Survival of patients with advanced colorectal cancer improves with the availability of fluorouracil-leucovorin, irinotecan, and oxaliplatin in the course of treatment. *J Clin Oncol* 2004;**22**:1209–14.
20. Koopman M, Antonini NF, Douma J, et al. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer [CAIRO]: a phase III randomised controlled trial. *Lancet* 2007;**370**:135–42.
21. Seymour MT, Maughan TS, Ledermann JA, et al. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer [MRC FOCUS]: a randomised controlled trial. *Lancet* 2007;**370**:143–52.
22. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;**351**:337–45.
23. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;**350**:2335–42.
24. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Eur J Cancer* 2005;**41**:1690–6.
25. Wohlhueter RM, McIvor RS, Plagemann PG. Facilitated transport of uracil and 5-fluorouracil, and permeation of orotic acid into cultured mammalian cells. *J Cell Physiol* 1980;**104**:309–19.
26. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003;**3**:330–8.
27. Walko CM, Lindley C. Capecitabine: a review. *Clin Ther* 2005;**27**:23–44.
28. van TB, Pinedo HM, Giaccone G, Peters GJ. Downstream molecular determinants of response to 5-fluorouracil and antifolate thymidylate synthase inhibitors. *Ann Oncol* 2000;**11**(4):385–91.
29. van Triest B, Pinedo HM, Blaauwgeers JL, et al. Prognostic role of thymidylate synthase, thymidine phosphorylase/platelet-derived endothelial cell growth factor, and proliferation markers in colorectal cancer. *Clin Cancer Res* 2000;**6**:1063–72.

30. Rich TA, Shepard RC, Mosley ST. Four decades of continuing innovation with fluorouracil: current and future approaches to fluorouracil chemoradiation therapy. *J Clin Oncol* 2004;**22**:2214–32.
31. Pinedo HM, Peters GF. Fluorouracil: biochemistry and pharmacology. *J Clin Oncol* 1988;**6**:1653–64.
32. Ichikawa W, Uetake H, Shirota Y, et al. Both gene expression for orotate phosphoribosyltransferase and its ratio to dihydropyrimidine dehydrogenase influence outcome following fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. *Br J Cancer* 2003;**89**:1486–92.
33. Ichikawa W, Uetake H, Shirota Y, et al. Combination of dihydropyrimidine dehydrogenase and thymidylate synthase gene expressions in primary tumors as predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. *Clin Cancer Res* 2003;**9**:786–91.
34. Grivicich I, Mans DR, Peters GJ, Schwartsmann G. Irinotecan and oxaliplatin: an overview of the novel chemotherapeutic options for the treatment of advanced colorectal cancer. *Braz J Med Biol Res* 2001;**34**:1087–103.
35. Chu E, Callender MA, Farrell MP, Schmitz JC. Thymidylate synthase inhibitors as anticancer agents: from bench to bedside. *Cancer Chemother Pharmacol* 2003;**52**(Suppl. 1):S80–9.
36. Grem JL, Fischer PH. Enhancement of 5-fluorouracil's anticancer activity by dipyridamole. *Pharmacol Ther* 1989;**40**:349–71.
37. Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil. *Clin Pharmacokinet* 1989;**16**:215–37.
38. Tabata T, Katoh M, Tokudome S, et al. Bioactivation of capecitabine in human liver: involvement of the cytosolic enzyme on 5'-deoxy-5-fluorocytidine formation. *Drug Metab Dispos* 2004;**32**:762–7.
39. Cao D, Russell RL, Zhang D, Leffert JJ, Pizzorno G. Uridine phosphorylase (–/–) murine embryonic stem cells clarify the key role of this enzyme in the regulation of the pyrimidine salvage pathway and in the activation of fluoropyrimidines. *Cancer Res* 2002;**62**:2313–7.
40. Takebayashi Y, Akiyama S, Akiba S, et al. Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. *J Natl Cancer Inst* 1996;**88**:1110–7.
41. Schuller J, Cassidy J, Dumont E, et al. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. *Cancer Chemother Pharmacol* 2000;**45**:291–7.
42. Nozawa T, Enomoto T, Koshida Y, Sato Y, Kuranami M. Specific enhanced expression of platelet-derived endothelial cell growth factor in submucosa of human colorectal cancer. *Dis Colon Rectum* 2004;**47**:2093–100.
43. Fox SB, Moghaddam A, Westwood M, et al. Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression in normal tissues: an immunohistochemical study. *J Pathol* 1995;**176**:183–90.
44. Beck A, Etienne MC, Cheradame S, et al. A role for dihydropyrimidine dehydrogenase and thymidylate synthase in tumour sensitivity to fluorouracil. *Eur J Cancer* 1994;**30**:1517–22.
45. Ciaparrone M, Quirino M, Schinzari G, et al. Predictive role of thymidylate synthase, dihydropyrimidine dehydrogenase and thymidine phosphorylase expression in colorectal cancer patients receiving adjuvant 5-fluorouracil. *Oncology* 2006;**70**:366–77.
46. Jensen SA, Vainer B, Sorensen JB. The prognostic significance of thymidylate synthase and dihydropyrimidine dehydrogenase in colorectal cancer of 303 patients adjuvantly treated with 5-fluorouracil. *Int J Cancer* 2007;**120**:694–701.
47. Kornmann M, Schwabe W, Sander S, et al. Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expression levels: predictors for survival in colorectal cancer patients receiving adjuvant 5-fluorouracil. *Clin Cancer Res* 2003;**9**:4116–24.
48. Lassmann S, Hennig M, Rosenberg R, et al. Thymidine phosphorylase, dihydropyrimidine dehydrogenase and thymidylate synthase mRNA expression in primary colorectal tumors-correlation to tumor histopathology and clinical follow-up. *Int J Colorectal Dis* 2006;**21**:238–47.
49. Oi K, Makino M, Ozaki M, et al. Immunohistochemical dihydropyrimidine dehydrogenase expression is a good prognostic indicator for patients with Dukes' C colorectal cancer. *Anticancer Res* 2004;**24**:273–9.
50. Soong R, Shah N, Salto-Tellez M, et al. Prognostic significance of thymidylate synthase, dihydropyrimidine dehydrogenase and thymidine phosphorylase protein expression in colorectal cancer patients treated with or without 5-fluorouracil-based chemotherapy. *Ann Oncol* 2008;**19**:915–9.
51. Westra JL, Hollema H, Schaapveld M, et al. Predictive value of thymidylate synthase and dihydropyrimidine dehydrogenase protein expression on survival in adjuvantly treated stage III colon cancer patients. *Ann Oncol* 2005;**16**:1646–53.
52. Meropol NJ, Gold PJ, Diasio RB, et al. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol* 2006;**24**:4069–77.
53. Yanagisawa Y, Maruta F, Iinuma N, et al. Modified Irinotecan/5FU/Leucovorin therapy in advanced colorectal cancer and predicting therapeutic efficacy by expression of tumor-related enzymes. *Scand J Gastroenterol* 2007;**42**:477–84.
54. Takenoue T, Kitayama J, Takei Y, et al. Characterization of dihydropyrimidine dehydrogenase on immunohistochemistry in colon carcinoma, and correlation between immunohistochemical score and protein level or messenger RNA expression. *Ann Oncol* 2000;**11**:273–9.
55. Tokunaga Y, Sasaki H, Saito T. Clinical role of orotate phosphoribosyl transferase and dihydropyrimidine dehydrogenase in colorectal cancer treated with postoperative fluoropyrimidine. *Surgery* 2007;**141**:346–53.
56. Tokunaga Y, Takahashi K, Saito T. Clinical role of thymidine phosphorylase and dihydropyrimidine dehydrogenase in colorectal cancer treated with postoperative fluoropyrimidine. *Hepatogastroenterology* 2005;**52**:1715–21.
57. Vallbohmer D, Kuramochi H, Shimizu D, et al. Molecular factors of 5-fluorouracil metabolism in colorectal cancer: analysis of primary tumor and lymph node metastasis. *Int J Oncol* 2006;**28**:527–33.
58. Vallbohmer D, Yang DY, Kuramochi H, et al. DPD is a molecular determinant of capecitabine efficacy in colorectal cancer. *Int J Oncol* 2007;**31**:413–8.
59. Koopman M, Venderbosch S, van Tinteren H, et al. Predictive and prognostic markers for the outcome of chemotherapy in advanced colorectal cancer, a retrospective analysis of the phase III randomized CAIRO study. *Eur J Cancer* 2009;**45**:1999–2006.
60. Schwartz PM, Moir RD, Hyde CM, Turek PJ, Handschumacher RE. Role of uridine phosphorylase in the anabolism of 5-fluorouracil. *Biochem Pharmacol* 1985;**34**:3585–9.
61. Mader RM, Sieder AE, Braun J, et al. Transcription and activity of 5-fluorouracil converting enzymes in fluoropyrimidine resistance in colon cancer in vitro. *Biochem Pharmacol* 1997;**54**:1233–42.
62. Ciccolini J, Evrard A, Cuq P. Thymidine phosphorylase and fluoropyrimidines efficacy: a Jekyll and Hyde story. *Curr Med Chem Anticancer Agents* 2004;**4**:71–81.

63. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990;**82**:4–6.
64. Matsumura M, Chiba Y, Lu C, et al. Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression correlated with tumor angiogenesis and macrophage infiltration in colorectal cancer. *Cancer Lett* 1998;**128**:55–63.
65. Matsuura T, Kuratate I, Teramachi K, Osaki M, Fukuda Y, Ito H. Thymidine phosphorylase expression is associated with both increase of intratumoral microvessels and decrease of apoptosis in human colorectal carcinomas. *Cancer Res* 1999;**59**:5037–40.
66. Saeki T, Tanada M, Takashima S, et al. Correlation between expression of platelet-derived endothelial cell growth factor (thymidine phosphorylase) and microvessel density in early-stage human colon carcinomas. *Jpn J Clin Oncol* 1997;**27**:227–30.
67. Takahashi Y, Bucana CD, Liu W, et al. Platelet-derived endothelial cell growth factor in human colon cancer angiogenesis: role of infiltrating cells. *J Natl Cancer Inst* 1996;**88**:1146–51.
68. Yasuno M, Mori T, Koike M, et al. Importance of thymidine phosphorylase expression in tumor stroma as a prognostic factor in patients with advanced colorectal carcinoma. *Oncol Rep* 2005;**13**:405–12.
69. Allegra CJ, Paik S, Colangelo LH, et al. Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with Dukes' B and C colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project collaborative study. *J Clin Oncol* 2003;**21**:241–50.
70. Berger SH, Jenh CH, Johnson LF, Berger FG. Thymidylate synthase overproduction and gene amplification in fluorodeoxyuridine-resistant human cells. *Mol Pharmacol* 1985;**28**:461–7.
71. Johnston PG, Drake JC, Trepel J, Allegra CJ. Immunological quantitation of thymidylate synthase using the monoclonal antibody TS 106 in 5-fluorouracil-sensitive and -resistant human cancer cell lines. *Cancer Res* 1992;**52**:4306–12.
72. Popat S, Matakidou A, Houlston RS. Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. *J Clin Oncol* 2004;**22**:529–36.
73. Aschele C, Debernardis D, Tunesi G, Maley F, Sobrero A. Thymidylate synthase protein expression in primary colorectal cancer compared with the corresponding distant metastases and relationship with the clinical response to 5-fluorouracil. *Clin Cancer Res* 2000;**6**:4797–802.
74. Bendardaf R, Elzagheid A, Lamlum H, et al. Thymidylate synthase expression in primary colorectal tumours is correlated with its expression in metastases. *Scand J Gastroenterol* 2007;**42**:471–6.
75. Benatti P, Gafa R, Barana D, et al. Microsatellite instability and colorectal cancer prognosis. *Clin Cancer Res* 2005;**11**:8332–40.
76. Elsaleh H, Iacopetta B. Microsatellite instability is a predictive marker for survival benefit from adjuvant chemotherapy in a population-based series of stage III colorectal carcinoma. *Clin Colorectal Cancer* 2001;**1**:104–9.
77. Gryfe R, Kim H, Hsieh ET, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 2000;**342**:69–77.
78. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005;**23**:609–18.
79. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003;**349**:247–57.
80. Raymond E, Chaney SG, Taamma A, Cvitkovic E. Oxaliplatin: a review of preclinical and clinical studies. *Ann Oncol* 1998;**9**:1053–71.
81. Lothe RA, Peltomaki P, Meling GI, et al. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res* 1993;**53**:5849–52.
82. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. *J Clin Oncol* 2003;**21**:1174–9.
83. Cunningham JM, Christensen ER, Tester DJ, et al. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. *Cancer Res* 1998;**58**:3455–60.
84. Kane MF, Loda M, Gaida GM, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997;**57**:808–11.
85. Mecklin JP. Frequency of hereditary colorectal carcinoma. *Gastroenterology* 1987;**93**:1021–5.
86. Samowitz WS, Curtin K, Ma KN, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:917–23.
87. Sankila R, Aaltonen LA, Jarvinen HJ, Mecklin JP. Better survival rates in patients with MLH1-associated hereditary colorectal cancer. *Gastroenterology* 1996;**110**:682–7.
88. Ward R, Meagher A, Tomlinson I, et al. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. *Gut* 2001;**48**:821–9.
89. Arnold CN, Goel A, Boland CR. Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. *Int J Cancer* 2003;**106**:66–73.
90. Carethers JM, Chauhan DP, Fink D, et al. Mismatch repair proficiency and in vitro response to 5-fluorouracil. *Gastroenterology* 1999;**117**:123–31.
91. Fink D, Nebel S, Aebi S, et al. The role of DNA mismatch repair in platinum drug resistance. *Cancer Res* 1996;**56**:4881–6.
92. Sergent C, Franco N, Chapusot C, et al. Human colon cancer cells surviving high doses of cisplatin or oxaliplatin in vitro are not defective in DNA mismatch repair proteins. *Cancer Chemother Pharmacol* 2002;**49**:445–52.
93. Magrini R, Bhonde MR, Hanski ML, et al. Cellular effects of CPT-11 on colon carcinoma cells: dependence on p53 and hMLH1 status. *Int J Cancer* 2002;**101**:23–31.
94. Koopman M, Kortman GA, Mekenkamp L, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. *Br J Cancer* 2009;**100**:266–73.
95. Carethers JM, Smith EJ, Behling CA, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004;**126**:394–401.
96. Sargent DJ, Marsoni S, Thibodeau SN, et al. Confirmation of deficient mismatch repair (dMMR) as a predictive marker for lack of benefit from 5-FU based chemotherapy in stages II and III colon cancer (CC): a pooled molecular reanalysis of randomized chemotherapy trials. *J Clin Oncol* 2008;**26**(Suppl.) [abstract 4008].
97. Duffy MJ, van Dalen A, Haglund C, et al. Tumour markers in colorectal cancer: European group on tumour markers ([EGTM) guidelines for clinical use. *Eur J Cancer* 2007;**43**:1348–60.
98. Locker GY, Hamilton S, Harris J, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 2006;**24**:5313–27.
99. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol* 2002;**20**:1043–8.

100. Rivory LP, Robert J. Molecular, cellular, and clinical aspects of the pharmacology of 20(S)camptothecin and its derivatives. *Pharmacol Ther* 1995;**68**:269–96.
101. Hsiang YH, Lihou MG, Liu LF. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res* 1989;**49**:5077–82.
102. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer* 2006;**6**:789–802.
103. Morton CL, Wadkins RM, Danks MK, Potter PM. The anticancer prodrug CPT-11 is a potent inhibitor of acetylcholinesterase but is rapidly catalyzed to SN-38 by butyrylcholinesterase. *Cancer Res* 1999;**59**:1458–63.
104. Rivory LP, Bowles MR, Robert J, Pond SM. Conversion of irinotecan (CPT-11) to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by human liver carboxylesterase. *Biochem Pharmacol* 1996;**52**:1103–11.
105. Rivory LP, Chatelut E, Canal P, Mathieu-Boue A, Robert J. Kinetics of the in vivo interconversion of the carboxylate and lactone forms of irinotecan (CPT-11) and of its metabolite SN-38 in patients. *Cancer Res* 1994;**54**:6330–3.
106. Kobayashi K, Bouscarel B, Matsuzaki Y, Ceryak S, Kudoh S, Fromm H. PH-dependent uptake of irinotecan and its active metabolite, SN-38, by intestinal cells. *Int J Cancer* 1999;**83**:491–6.
107. Mathijssen RH, van Alphen RJ, Verweij J, et al. Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin Cancer Res* 2001;**7**:2182–94.
108. Combes O, Barre J, Duche JC, et al. In vitro binding and partitioning of irinotecan [CPT-11] and its metabolite, SN-38, in human blood. *Invest New Drugs* 2000;**18**:1–5.
109. Burke TG, Munshi CB, Mi Z, Jiang Y. The important role of albumin in determining the relative human blood stabilities of the camptothecin anticancer drugs. *J Pharm Sci* 1995;**84**:518–9.
110. Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I. Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. *Drug Metab Dispos* 2005;**33**:434–9.
111. Humerickhouse R, Lohrbach K, Li L, Bosron WF, Dolan ME. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. *Cancer Res* 2000;**60**:1189–92.
112. Wu MH, Yan B, Humerickhouse R, Dolan ME. Irinotecan activation by human carboxylesterases in colorectal adenocarcinoma cells. *Clin Cancer Res* 2002;**8**:2696–700.
113. Satoh T, Hosokawa M. Structure, function and regulation of carboxylesterases. *Chem Biol Interact* 2006;**162**: 195–211.
114. Iyer L, King CD, Whitington PF, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 1998;**101**:847–54.
115. Gagne JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C. Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Mol Pharmacol* 2002;**62**:608–17.
116. Santos A, Zanetta S, Cresteil T, et al. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. *Clin Cancer Res* 2000;**6**:2012–20.
117. Dodds HM, Haaz MC, Riou JF, Robert J, Rivory LP. Identification of a new metabolite of CPT-11 (irinotecan): pharmacological properties and activation to SN-38. *J Pharmacol Exp Ther* 1998;**286**:578–83.
118. Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD. Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. *Drug Resist Updat* 2003;**6**:71–84.
119. Boonsong A, Curran S, McKay JA, Cassidy J, Murray GI, McLeod HL. Topoisomerase I protein expression in primary colorectal cancer and lymph node metastases. *Hum Pathol* 2002;**33**:1114–9.
120. Paradiso A, Xu J, Mangia A, et al. Topoisomerase-I, thymidylate synthase primary tumour expression and clinical efficacy of 5-FU/CPT-11 chemotherapy in advanced colorectal cancer patients. *Int J Cancer* 2004;**111**:252–8.
121. Jansen WJ, Zwart B, Hulscher ST, Giaccone G, Pinedo HM, Boven E. CPT-11 in human colon-cancer cell lines and xenografts: characterization of cellular sensitivity determinants. *Int J Cancer* 1997;**70**:335–40.
122. Vallbohmer D, Iqbal S, Yang DY, et al. Molecular determinants of irinotecan efficacy. *Int J Cancer* 2006;**119**:2435–42.
123. Braun MS, Richman SD, Quirke P, et al. Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. *J Clin Oncol* 2008;**26**:2690–8.
124. Guichard S, Terret C, Hennebelle I, et al. CPT-11 converting carboxylesterase and topoisomerase activities in tumour and normal colon and liver tissues. *Br J Cancer* 1999;**80**:364–70.
125. Sanghani SP, Quinney SK, Fredenburg TB, et al. Carboxylesterases expressed in human colon tumor tissue and their role in CPT-11 hydrolysis. *Clin Cancer Res* 2003;**9**:4983–91.
126. Xu G, Zhang W, Ma MK, McLeod HL. Human carboxylesterase 2 is commonly expressed in tumor tissue and is correlated with activation of irinotecan. *Clin Cancer Res* 2002;**8**:2605–11.
127. Mathe G, Kidani Y, Segiguchi M, et al. Oxalato-platinum or 1-OHP, a third-generation platinum complex: an experimental and clinical appraisal and preliminary comparison with cis-platinum and carboplatinum. *Biomed Pharmacother* 1989;**43**:237–50.
128. Di Francesco AM, Ruggiero A, Riccardi R. Cellular and molecular aspects of drugs of the future: oxaliplatin. *Cell Mol Life Sci* 2002;**59**:1914–27.
129. Misset JL, Bleiberg H, Sutherland W, Bekradda M, Cvitkovic E. Oxaliplatin clinical activity: a review. *Crit Rev Oncol Hematol* 2000;**35**:75–93.
130. Kweekel DM, Gelderblom H, Guchelaar HJ. Pharmacology of oxaliplatin and the use of pharmacogenomics to individualize therapy. *Cancer Treat Rev* 2005;**31**:90–105.
131. Jerremalm E, Wallin I, Yachnin J, Ehrsson H. Oxaliplatin degradation in the presence of important biological sulphur-containing compounds and plasma ultrafiltrate. *Eur J Pharm Sci* 2006;**28**:278–83.
132. Allain P, Heudi O, Cailleux A, et al. Early biotransformations of oxaliplatin after its intravenous administration to cancer patients. *Drug Metab Dispos* 2000;**28**:1379–84.
133. Graham MA, Lockwood GF, Greenslade D, Brienza S, Bayssas M, Gamelin E. Clinical pharmacokinetics of oxaliplatin: a critical review. *Clin Cancer Res* 2000;**6**:1205–18.
134. Raymond E, Faivre S, Chaney S, Woynarowski J, Cvitkovic E. Cellular and molecular pharmacology of oxaliplatin. *Mol Cancer Ther* 2002;**1**:227–35.
135. Faivre S, Chan D, Salinas R, Woynarowska B, Woynarowski JM. DNA strand breaks and apoptosis induced by oxaliplatin in cancer cells. *Biochem Pharmacol* 2003;**66**:225–37.
136. Viguier J, Boige V, Miquel C, et al. ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. *Clin Cancer Res* 2005;**11**:6212–7.

137. Zhang S, Lovejoy KS, Shima JE, et al. Organic cation transporters are determinants of oxaliplatin cytotoxicity. *Cancer Res* 2006;**66**:8847–57.
138. Yonezawa A, Masuda S, Yokoo S, Katsura T, Inui K. Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1–3 and multidrug and toxin extrusion family). *J Pharmacol Exp Ther* 2006;**319**:879–86.
139. Holzer AK, Manorek GH, Howell SB. Contribution of the major copper influx transporter CTR1 to the cellular accumulation of cisplatin, carboplatin, and oxaliplatin. *Mol Pharmacol* 2006;**70**:1390–4.
140. Safaei R, Howell SB. Copper transporters regulate the cellular pharmacology and sensitivity to Pt drugs. *Crit Rev Oncol Hematol* 2005;**53**:13–23.
141. de Laat WL, Jaspers NG, Hoeijmakers JH. Molecular mechanism of nucleotide excision repair. *Genes Dev* 1999;**13**:768–85.
142. Arnould S, Hennebelle I, Canal P, Bugat R, Guichard S. Cellular determinants of oxaliplatin sensitivity in colon cancer cell lines. *Eur J Cancer* 2003;**39**:112–9.
143. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med* 2006;**355**:983–91.
144. Viguier J, Boige V, Miquel C, et al. ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. *Clin Cancer Res* 2005;**11**:6212–7.
145. Shirota Y, Stoehlmacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin Oncol* 2001;**19**:4298–304.
146. Calascibetta A, Cabibi D, Martorana A, et al. Thymidylate synthase gene promoter polymorphisms are associated with TSmRNA expressions but not with microsatellite instability in colorectal cancer. *Anticancer Res* 2004;**24**:3875–80.
147. Takechi T, Okabe H, Fujioka A, Murakami Y, Fukushima M. Relationship between protein levels and gene expression of dihydropyrimidine dehydrogenase in human tumor cells during growth in culture and in nude mice. *Jpn J Cancer Res* 1998;**89**:1144–53.
148. Inokuchi M, Uetake H, Shirota Y, Yamada H, Tajima M, Sugihara K. Gene expression of 5-fluorouracil metabolic enzymes in primary colorectal cancer and corresponding liver metastasis. *Cancer Chemother Pharmacol* 2004;**53**:391–6.
149. Kuramochi H, Hayashi K, Uchida K, et al. 5-Fluorouracil-related gene expression levels in primary colorectal cancer and corresponding liver metastasis. *Int J Cancer* 2006;**119**:522–6.
150. Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007;**449**:557–63.
151. Kim BG, Li C, Qiao W, et al. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. *Nature* 2006;**441**:1015–9.
152. Saito S, Tsuno N, Nagawa H, et al. Expression of platelet-derived endothelial cell growth factor correlates with good prognosis in patients with colorectal carcinoma. *Cancer* 2000;**88**:42–9.
153. Kyzas PA, axa-Kyza D, Ioannidis JP. Almost all articles on cancer prognostic markers report statistically significant results. *Eur J Cancer* 2007;**43**:2559–79.
154. Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. *Clin Cancer Res* 2008;**14**:5967–76.
155. Taylor JM, Ankerst DP, Andridge RR. Validation of biomarker-based risk prediction models. *Clin Cancer Res* 2008;**14**:5977–83.
156. de Bono JS, Twelves CJ. The oral fluorinated pyrimidines. *Invest New Drugs* 2001;**19**:41–59.
157. Johnston PG, Kaye S. Capecitabine: a novel agent for the treatment of solid tumors. *Anticancer Drugs* 2001;**12**:639–46.
158. Miwa M, Ura M, Nishida M, et al. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. *Eur J Cancer* 1998;**34**:1274–81.
159. Urien S, Rezai K, Lokiec F. Pharmacokinetic modelling of 5-FU production from capecitabine – a population study in 40 adult patients with metastatic cancer. *J Pharmacokinet Pharmacodyn* 2005;**32**:817–33.
160. Lamont EB, Schilsky RL. The oral fluoropyrimidines in cancer chemotherapy. *Clin Cancer Res* 1999;**5**:2289–96.
161. Leichman CG, Lenz HJ, Leichman L, et al. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. *J Clin Oncol* 1997;**15**:3223–9.
162. Lenz HJ, Hayashi K, Salonga D, et al. P53 point mutations and thymidylate synthase messenger RNA levels in disseminated colorectal cancer: an analysis of response and survival. *Clin Cancer Res* 1998;**4**:1243–50.
163. Aschele C, Debernardis D, Casazza S, et al. Immunohistochemical quantitation of thymidylate synthase expression in colorectal cancer metastases predicts for clinical outcome to fluorouracil-based chemotherapy. *J Clin Oncol* 1999;**17**:1760–70.
164. Edler D, Glimelius B, Hallstrom M, et al. Thymidylate synthase expression in colorectal cancer: a prognostic and predictive marker of benefit from adjuvant fluorouracil-based chemotherapy. *J Clin Oncol* 2002;**20**:1721–8.
165. Allegra CJ, Parr AL, Wold LE, et al. Investigation of the prognostic and predictive value of thymidylate synthase, p53, and Ki-67 in patients with locally advanced colon cancer. *J Clin Oncol* 2002;**20**:1735–43.
166. Johnston PG, Benson 3rd AB, Catalano P, Rao MS, O'Dwyer PJ, Allegra CJ. Thymidylate synthase protein expression in primary colorectal cancer: lack of correlation with outcome and response to fluorouracil in metastatic disease sites. *J Clin Oncol* 2003;**21**:815–9.
167. Bendardaf R, Lamlum H, Elzagheid A, Ristamaki R, Pyrhonen S. Thymidylate synthase expression levels: a prognostic and predictive role in advanced colorectal cancer. *Oncol Rep* 2005;**14**:657–62.
168. Popat S, Chen Z, Zhao D, et al. A prospective blinded analysis of thymidylate synthase and p53 expression as prognostic markers in the adjuvant treatment of colorectal cancer. *Ann Oncol* 2006;**17**:1820–7.
169. Smith NF, Figg WD, Sparreboom A. Pharmacogenetics of irinotecan metabolism and transport: an update. *Toxicol In Vitro* 2006;**20**:163–75.
170. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer* 2006;**6**:789–802.
171. Gelderblom HA, de Jonge MJ, Sparreboom A, Verweij J. Oral topoisomerase 1 inhibitors in adult patients: present and future. *Invest New Drugs* 1999;**17**:401–15.
172. Garcia-Carbonero R, Supko JG. Current perspectives on the clinical experience, pharmacology, and continued development of the camptothecins. *Clin Cancer Res* 2002;**8**:641–61.
173. Cummings J, Boyd G, Macpherson JS, et al. Factors influencing the cellular accumulation of SN-38 and camptothecin. *Cancer Chemother Pharmacol* 2002;**49**:194–200.

174. Chaney SG, Vaisman A. Specificity of platinum-DNA adduct repair. *J Inorg Biochem* 1999;**77**:71–81.
175. Gamelin E, Bouil AL, Boisdron-Celle M, et al. Cumulative pharmacokinetic study of oxaliplatin, administered every three weeks, combined with 5-fluorouracil in colorectal cancer patients. *Clin Cancer Res* 1997;**3**:891–9.
176. Ghezzi A, Aceto M, Cassino C, Gabano E, Osella D. Uptake of antitumor platinum[II]-complexes by cancer cells, assayed by inductively coupled plasma mass spectrometry [ICP-MS]. *J Inorg Biochem* 2004;**98**:73–8.
177. Luo FR, Wyrick SD, Chaney SG. Biotransformations of oxaliplatin in rat blood in vitro. *J Biochem Mol Toxicol* 1999;**13**:159–69.
178. Raymond E, Faivre S, Woynarowski JM, Chaney SG. Oxaliplatin: mechanism of action and antineoplastic activity. *Semin Oncol* 1998;**25**:4–12.
179. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* 2005;**4**:307–20.