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TOPIC HIGHLIGHT

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FOLFOX/FOLFIRI pharmacogenetics: The call for a personalized approach in colorectal cancer therapy

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Abstract

While 5-fluorouracil used as single agent in patients with metastatic colorectal cancer has an objective response rate around 20%, the administration of combinations of irinotecan with 5-fluorouracil/folinic acid or oxaliplatin with 5-fluorouracil/folinic acid results in significantly increased response rates and improved survival. However, the side effects of systemic therapy such as myelotoxicity, neurotoxicity or gastrointestinal toxicity may lead to life-threatening complications and have a major impact on the quality of life of the patients. Therefore, biomarkers that would be instrumental in the choice of optimal type, combination and dose of drugs for an individual patient are urgently needed. The efficacy and toxicity of anticancer drugs in tumor cells is determined by the effective concentration in tumor cells, healthy tissues and by the presence and quantity of the drug targets. Enzymes active in drug metabolism and transport represent important determinants of the therapeutic outcome. The aim of this review was to summarize published data on associations of gene and protein expression, and genetic variability of putative biomarkers with response to therapy of colorectal cancer to 5-fluorouracil/leucovorin/oxaliplatin and 5-fluorouracil/leukovorin/irinotecan regimens. Gaps in the knowledge identified by this review may aid the design of future research and clinical trials.

Key words: Colorectal cancer; Chemotherapy; 5-Fluorouracil; Oxaliplatin; Irinotecan

Core tip: 5-fluorouracil/leucovorin combined with oxaliplatin (FOLFOX) and irinotecan (FOLFIRI) represent the most effective chemotherapy regimens for colorectal carcinoma patients with distant metastases. Pharmacogenetics represents a promising strategy for the individualization of therapy, including identification of patients at increased risk of toxicity. This review summarizes contemporary knowledge about associations of gene and protein expression and genetic variability of putative biomarkers for the response of colorectal cancer to FOLFOX/FOLFIRI regimens. From the published data reviewed it is obvious that the problem is highly complex and the ultimate profile of the drug-sensitive or resistant patient will most probably be jointly defined by genetic, epigenetic, intracellular, extracellular, and extrinsic factors.

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INTRODUCTION

Colorectal carcinoma is one of the most common causes of cancer mortality. While localized tumors are amenable to curative surgical resection, the curative potential of surgery in patients with metastatic disease is limited. Intuitively, the best approach to treat metastatic (systemic) disease is the systemic administration of therapy. Systemic therapy may also be used as adjuvant treatment after the resection of primary tumors in patients who have no evidence of metastatic disease, but are suspected to harbor microscopic metastases. Among the modalities of systemic treatment, chemotherapy has been the most widely used in patients with colorectal carcinoma, and in recent years several targeted agents have complemented the therapeutic armamentarium in patients with metastatic colorectal carcinoma.

Over the past half-century, fluoropyrimidines have constituted the backbone of chemotherapeutic regimens in colorectal carcinoma. Early randomized clinical trials demonstrated that the administration of fluoropyrimidine-based chemotherapy results in statistically significant prolongation of survival in patients with metastatic colorectal carcinoma[1,2]. Among fluoropyrimidines, 5-fluorouracil (5-FU) has been the most commonly used agent. Prospective studies have demonstrated the benefit of the administration of 5-FU in an infusional regimen and in combination with folinic acid[3]. In addition, in patients with isolated liver metastases the benefit of hepatic arterial infusion of fluoropyrimidines has also been demonstrated[4].

The next generation of regimens for the treatment of metastatic colorectal carcinoma has been introduced with the advent of two cytotoxic agents, irinotecan, a topoisomerase I inhibitor, and oxaliplatin, a platinum derivative, in the late 1990s. The activity of irinotecan and oxaliplatin was first demonstrated in patients that failed on fluoropyrimidines. While 5-FU alone has an objective response rate around 20%[3,5], the combinations of irinotecan with 5-FU/folinic acid and oxaliplatin with 5-FU/folinic acid result in significantly increased response rates and improved survival[6-8]. The activity of 5-FU/leucovorin combined with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) in the first-line treatment of metastatic colorectal carcinoma is comparable[9].

Another major step forward in the systemic management of colorectal carcinoma was the advent of monoclonal antibodies targeting vascular endothelial growth factor (VEGF) pathway or epidermal growth factor receptor (EGFR)[10-14]. Currently available targeted agents active in metastatic colorectal carcinoma include the anti-VEGF antibody bevacizumab, the anti-EGFR antibodies cetuximab and panitumumab, and the anti-VEGF agents aflibercept and regorafenib. These agents are active, mostly in combination with cytotoxic drugs, both in the first-line of therapy as well as in previously treated patients with metastatic colorectal cancer.

Besides causing morbidity and occasional mortality, the side effects of systemic therapy have a major impact on the quality of life of the patients. Common to fluoropyrimidines, irinotecan and oxaliplatin is myelotoxicity, the administration of fluoropyrimidines and irinotecan is frequently accompanied by gastrointestinal toxicity[15], while neurotoxicity regularly complicates the administration of oxaliplatin[16,17]. The administration of targeted agents is also not devoid of side effects that may be very annoying, *e.g.*, skin toxicity associated with the administration of anti-EGFR agents[18] or hypertension after anti-VEGF therapy[11]. Liver toxicities that accompany the administration of agents used in colorectal carcinoma encompass non-alcoholic fatty liver disease after 5-FU, sinusoid obstruction syndrome after oxaliplatin or steatohepatitis after irinotecan[19]. These toxicities could result in postoperative complications in patients undergoing subsequent liver resection[19].

The role of pharmacogenetics in the management of patients with colorectal carcinoma has long been neglected. The significance of genetic polymorphisms of enzymes responsible for fluoropyrimidine degradation, *e.g.*, dihydropyrimidine dehydrogenase, has been known for some time, but the assessment of these biomarkers has still not found routine use[20]. With the advent of targeted therapy, the presence or absence of RAS mutations has been identified as a predictive biomarker of efficacy of anti-EGFR antibodies[21].

PHARMACOGENETICS OF FOLFOX/FOLFIRI REGIMENS IN COLORECTAL CANCER

In general, the use of chemotherapy to treat cancers is limited by the inter-individual variability in drug response and by the development of resistance. Anticancer drugs are metabolized predominantly in liver, subsequently transported in conjugated or unconjugated form to the tumor microenvironment, where drug uptake/efflux transporters modulate intracellular levels of the drug or its active metabolites. The efficacy of anticancer drugs in tumor cells is dependent on the effective concentrations and on the presence and quantity of the drug targets. There are marked inter-individual differences in expression levels and activities of enzymes modifying efficacy, as well as toxicity, of anticancer drugs. From this point of view it seems obvious that biomarkers enabling prediction of optimal type, combination and dose of drugs for each patient may exist, and their use for individualization of therapy is envisaged. Such individualization would be highly cost-effective and socially desirable because of the prolonged survival and improved quality of life of large number of cancer patients.

However, this task is very complicated because of numerous factors determining the final functional phenotype of enzymes responsible for the drug metabolism, transport and targets. On the intracellular level, genotype *vs* phenotype relations must be considered along with

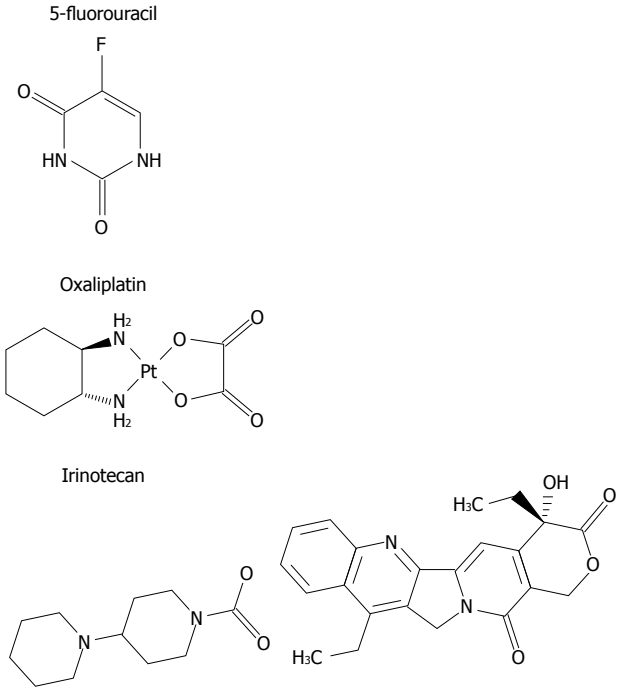

Figure 1 Chemical structures of components of 5-fluorouracil/leucovorin combined with oxaliplatin and irinotecan regimens.

epigenetic factors, such as methylation of regulatory DNA elements, histone acetylation, the presence of micro RNAs and other non-coding RNA species, protein-protein and DNA/RNA-protein interactions. Extracellular factors may include immune response or hormonal balance that could determine the expression pattern of intracellular enzymes, drug-drug and drug-environmental/alimentary interactions and other as yet unknown factors. Last, but not least, enzymes responding to drug administration may be induced to a high extent by repeated doses of the drug and the study of this phenomenon *in vivo* is very difficult. Investigation of such a complex system where the roles of a number of factors remain unknown is significantly limited by the currently available equipment and empirical approaches. Thus, from many published studies, very few of the most pertinent biomarkers emerged, which should be verified by upcoming controlled prospective clinical trials. This review summarizes the most promising predictive biomarkers for FOLFOX/FOLFIRI regimens in advanced colorectal cancers and highlights potential research trends.

5-FU

5-FU belongs to fluoropyrimidine drugs (Figure 1), which are widely used in the therapy of gastrointestinal cancers including colorectal cancer. Research on 5-FU pharmacogenetics (and pharmacogenomics) focused mainly on interindividual differences in 5-FU pharmacokinetics and genetic alterations in genes encoding transmembrane transporters and 5-FU-metabolizing enzymes, such as dihydropyrimidine dehydrogenase (DPD/DPYD, OMIM: 612779), thymidine phosphorylase (TYMP, OMIM: 131222), thymidine kinase 1 (TK1, OMIM: 188300), uridine monophosphate synthetase (UMPS/OPRT, OMIM: 613891)[22] (Table 1).

A low accumulation of 5-FU in cancer cells may be caused by altered membrane transport, namely drug efflux, mediated mainly by ATP-binding cassette (ABC) transporters, or reduced drug uptake into the cancer cells, mediated mainly by solute carrier (SLC) transporters[23,24].

5-FU is phosphorylated to FdUMP by TYMP and TK1. FdUMP then inhibits an important enzyme for nucleotide synthesis - thymidylate synthase (TYMS, OMIM: 188350)[22]. 5-FU is also indirectly phosphorylated by UMPS *via* fluorouridine monophosphate (FUMP) to fluorouridine diphosphate (FUDP) and then converted by ribonucleotide reductase (RRM1 and 2, OMIM: 180410, OMIM: 180390, respectively) to FdUMP. The nucleotide diphosphate kinase (NME1/NM23, OMIM: 156490)-formed fluorodeoxyuridine triphosphate (FdUTP) incorporates into DNA and fluorouridine triphosphate (FUTP) incorporates into RNA and both cause chain termination[25]. It appears that the spectrum of 5-FU metabolites depends on the administration schedule, as bolus treatment favors RNA damage by FUTP and continuous regimen favors DNA damage by FdUTP[26]. DPYD catalyzes inactivation of 5-FU into inactive dihydrofluorouracil, mostly in the liver[27].

Biomarkers of 5-FU chemoresistance in colorectal cancer

Deregulation of ABC transporters in CRC tumors compared to non-malignant colon tissue has been reported recently[28]. Interestingly, the best-known ABC transporter, ABCB1 coding P-glycoprotein (OMIM: 171050), has not been shown to modify the sensibility of human-derived esophageal carcinoma cell lines to 5-FU[29]. Moreover, lack of relationship between the ABCB1 protein or transcript expression, genotype and long-term prognosis of patients treated by 5-FU was reported[28,30]. Transporters from the ABCC family can collectively confer resistance to anticancer drugs and their conjugated metabolites, platinum compounds, folate antimetabolites, nucleoside and nucleotide analogues *in vitro*[31]. In particular, the expression of ABCC2 (OMIM: 601107), ABCC3 (OMIM: 604323), ABCC4 (OMIM: 605250), ABCC5 (OMIM: 605251), ABCC6 (OMIM: 603234) and ABCC11 (OMIM: 607040) induced resistance to 5-FU *in vitro*[29,32-34]. Nevertheless, the results obtained using cell line models treated by the studied drug for a long time may not reflect the real situation in such a heterogeneous entity as a colorectal tumor. In breast cancer patients treated with neoadjuvant chemotherapy, ABCA1 (OMIM: 600046), ABCA12 (OMIM: 607800), ABCB6 (OMIM: 605452), ABCC5, ABCC11 and ABCC13 (OMIM: 608835) transcript levels were downregulated in patients with a complete pathological response compared with patients with residual disease[35]. Oguri *et al*[36] discovered that ABCC11 expression is induced by 5-FU and that ABCC11 is directly involved

Table 1 Putative biomarkers of efficacy and/or toxicity of 5-fluorouracil in colorectal cancer

Gene	Transcript	Protein	SNP	Effect	Ref.
SLC29A1	-	High	-	Inferior clinical response	Phua *et al*[40] 2013
ABCC11	Low	-	-	Inferior response and shorter DFS	Hlavata *et al*[28] 2012
TYMP	High	High	-	Longer DFS and OS	Meropol *et al*[48] 2006
		High	-	Better clinical response	Sadahiro *et al*[51] 2012
	-	High cytoplasmic	-	Longer OS	Mitselou *et al*[52] 2012
	High	-	-	pCR	Chiorean *et al*[50] 2012
	-	No association	-	DFS and OS	Ciaparrone *et al*[42] 2006
	No association	-	-	OS	Lassmann *et al*[43] 2006
	-	No association	-	OS	Soong *et al*[44] 2008
	No association	-	-	Clinical response	Vallbohmer *et al*[45] 2007
	No association	-	-	PFS and OS	Koopman *et al*[49] 2009
UMPS	No association	-	-	Longer OS	Yanagisawa *et al*[54] 2007
	-	High	-	Longer OS	Tokunaga *et al*[55] 2007
	-	High (tumor cells)	-	Shorter OS	Koopman *et al*[56] 2009
	-	High (stromal cells)	-	Longer OS	Koopman *et al*[56] 2009
DPYD	Low	-	-	Longer OS	Yanagisawa *et al*[54] 2007
	Low	-	-	Longer OS	Vallböhmer *et al*[45] 2007
	-	Low	-	Longer OS	Tokunaga *et al*[55] 2007
	-	Low[1]	-	Longer OS[1]	Koopman *et al*[56] 2009
	-	Low	-	Longer OS	Ciaparrone *et al*[42] 2007
	-	No association	-	DFS	Westra *et al*[59] 2005
	-	-	Rs3918290	Higher toxicity	Caudle *et al*[27] 2013
				Dose reduction recommended	Swen *et al*[62] 2011
	-	-	Rs55886062	Higher toxicity	Caudle *et al*[27] 2013
				Dose reduction recommended	Swen *et al*[62] 2011
	-	-	Rs67376798	Higher toxicity	Caudle *et al*[27] 2013
				Dose reduction recommended	Swen *et al*[62] 2011
TYMS	-	No association	-	Clinical response	Jennings *et al*[57] 2012
	-	-	Rs45445694	Lower protein expression, clinical benefit and higher toxicity improved	Jennings *et al*[57] 2012

[1]Only when combined with irinotecan. PFS: Progression-free survival; DFS: Disease-free survival; OS: Overall survival; pCR: Complete pathological response.

in resistance by the efflux transport of the active metabolite FdUMP in human small-cell lung cancer cell lines *in vitro*. High expression of ABCC11 has been associated with significantly better response and longer disease-free interval in colorectal cancer patients treated by first line 5-FU-based chemotherapy in either the palliative or adjuvant setting[28].

In humans, there are two major families of SLC transporters that transport nucleoside analogs including 5-FU: SLC28A (human concentrative transporters, namely SLC28A1, SLC28A2 and SLC28A3, OMIM: 606207, 606208, 608269, respectively) and SLC29A (human equilibrative transporters, namely SLC29A1, SLC29A2, SLC29A3 and SLC29A4, OMIM: 602193, 602110, 612373, 609149, respectively)[37,38]. A pilot study has indicated that colorectal tissue specimens from tumors that were resistant to 5-FU in an *in vitro* cell viability assay had higher expression of SLC29A1 mRNA[39]. These data were recently corroborated by another study showing correlation between high pre-treatment intratumoral SLC29A1 protein levels with worse clinical response to 5-FU[40]. The predictive significance of SLC29A1 has been studied extensively in pancreatic cancer. In contrast to colorectal cancer, the majority of studies on patients with pancreatic cancer have suggested that high SLC29A1 expression may be predictive of improved survival in patients treated with gemcitabine, but not for patients treated by 5-FU[41].

The issue of predictive value of phenotype and/or genotype of drug transporters remains open, mainly because of the complex nature of this phenomenon. No study investigated either the balance between uptake/efflux (status of both SLC and ABC transporters in the same cohort of patients) or the relationship between of drug transport and subsequent 5-FU metabolism and/or targets. The majority of available studies addressed single or isolated groups of biomarkers and contradictory results were often obtained.

Inside the tumor cells, 5-FU is converted to its monophosphate by TYMP, an angiogenic factor also known as platelet-derived endothelial cell growth factor (PD-ECGF). The question is whether TYMP acts as a predictor of poor prognosis because of its neoangiogenic activity in tumors or whether it may predict good prognosis because of activation of 5-FU. This dual role of TYMP may be one of the reasons for contradictory results of studies aiming to evaluate the role of TYMP as a prognostic biomarker. No association has been found in smaller studies with colorectal cancer patients treated by adjuvant 5-FU therapy[42-45]. TYMP protein expression also was not associated with disease-free survival and overall survival of the advanced colorectal cancer patients in other studies[46,47]. On the other hand, look-

ing at the studies with capecitabine, high expression of TYMP mRNA and protein was associated with longer overall and disease-free survival in patients with advanced colorectal cancer treated by capecitabine plus irinotecan as in the first-line setting[48]. In 566 advanced colorectal cancer patients treated with capecitabine, irinotecan and oxaliplatin enrolled in the phase Ⅲ CAIRO study, TYMP was neither a predictive nor prognostic factor[49]. In a phase Ⅱ trial of neoadjuvant capecitabine plus irinotecan and radiation therapy for locally advanced rectal cancer (n = 22 patients), high tumor TYMP transcript expression was associated with complete pathological response[50]. Similarly, right-sided colon tumors with high TYMP transcript levels had higher response rate to neoadjuvant chemotherapy with oral fluoropyrimidine tegafur[51]. TYMP protein expression has been observed not only in tumor cells, but also in the stroma and in endothelium and tumor-associated macrophages[52]. Previously, higher TYMP expression was reported in stromal and tumor cells compared with normal tissue[53]. TYMP expression levels in the cytoplasm of tumor cells and stromal cells correlated with vascular endothelial growth factor (VEGF, OMIM: 192240) expression by tumor cells and vessels. However only high cytoplasmic expression of TYMP was associated with longer overall survival of these patients[52]. This study further supported the view concept of a differential role of TYMP in tumor cells compared with the stroma.

The reported data regarding UMPS and uridine phosphorylase (UPP1, OMIM: 191730) are again conflicting. UMPS transcript levels did not correlate with overall survival of colorectal cancer patients[54], but Tokunaga *et al*[55] reported that high expression of the UMPS protein is associated with longer overall survival of patients with advanced colorectal cancer. On the other hand, high expression of UMPS in tumor cells was an unfavorable prognostic parameter for overall survival in the CAIRO study; however, the opposite effect was observed in stromal cells, with high stromal cell UMPS expression was being associated with favorable prognosis[54]. Moreover, UPP1 bypasses UMPS and is suggested to be the key enzyme in conversion of 5-FU to the active metabolite FUMP[56].

It becomes obvious that further studies on the role of metabolic pathway of 5-FU in the treatment outcome of patients should also consider the localization of biomarker expression within the various cell types and even intracellular components.

A recent meta-analysis of 39 studies that investigated 2402 patients for the most commonly studied polymorphic biomarkers *TYMS* (rs45445694) and *MTHFR* (rs1801133) revealed that the *TYMS* polymorphism is significantly associated with protein expression, clinical benefit and adverse effects[57]. However, the authors concluded that the association between treatment effect and *TYMS* genotype and subsequently protein level is so small that it is of limited clinical relevance.

5-FU is deactivated mainly by DPYD, and this enzyme has also been shown to be an independent chemosensitivity predictive factor *in vitro*[58]. Low expression of both DPYD mRNA[54] and protein[42,47,55] was associated with longer overall survival or disease free survival in numerous studies on colorectal cancer patients treated by 5-FU in the adjuvant or advanced setting. Soong *et al*[44] observed only a trend of shorter overall survival in patients with low DPYD protein expression. On the other hand, no association of DPYD mRNA or protein levels with disease-free or overall survival was observed in other studies[45,59]. Retrospective analysis of DPYD protein expression in well-defined groups of patients in the phase Ⅲ randomized CAIRO study did not confirm the predictive value of DPYD[49], except for the subgroup of patients treated with the combination of capecitabine plus irinotecan.

The B-CAST multicenter, prospective cohort study aims to analyze TYMP, DPYD and UMPS in a group of more than 2000 patients with stage Ⅲ colon cancer treated with adjuvant 5-FU-based regimens. The results of this first prospective clinical trial studying predictive biomarkers of 5-FU will hopefully define the effect of these three enzymes on treatment outcome[60].

Biomarkers of 5-FU toxicity in colorectal cancer patients

The expression of genes involved in 5-FU metabolism mentioned above may affect not only drug efficacy and resistance, but also toxicity (Table 1). However, data about the majority of these genes and their association with 5-FU toxicity are inconsistent and cannot be applied in clinical practice. The only exception is DPYD, for which evidence for the dosing recommendations comes from two large prospective studies, small studies and case studies[27]. Patients with DPYD deficiency treated with fluoropyrimidines suffered from severe toxicity, including mucositis and diarrhea, myelosuppression, neurotoxicity and hand-food syndrome[61]. The United States Food and Drug Administration (FDA) has added statements to the drug labels for 5-FU that contraindicate its use in DPYD enzyme deficient individuals. The Dutch Pharmacogenetics Working group and Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends the use of alternative drugs for heterozygous carriers of a decreased-activity allele[27,62] such as *DPYD*2A* (rs3918290), *DPYD*13* (rs55886062) or rs67376798. For heterozygous carriers, it is recommended to start with at least a 50% reduction of initial dose and re-adjust dosing according to patient's tolerability or pharmacokinetic tests[27]. Perhaps because of a number of other so far uncharacterized SNPs in *DPYD*, the presence of these variants does not always result in toxicity and the results of available studies are not consistent and have not been replicated.

In summary, the positive predictive value of *DPYD*2A* and *DPYD*13* variants to predict a severe toxicity (grade Ⅲ and Ⅳ) is 62% and negative predictive value is 95%[63,64]. Thus, the absence of these *DPYD* variants does not eliminate the risk of high-grade toxicity caused by additional rare variants in *DPYD* or from other factors including genetic, epigenetic, environmental or alimentary

factors. Moreover, the combination of 5-FU with other anticancer drugs or bolus administration of 5-FU may further increase the risk of severe toxicity in heterozygous carriers[27,64].

The individual risk/benefit ratio should be estimated for all patients because for some patients, the potential benefit may still exceed clinically tolerable toxicity.

OXALIPLATIN

Oxaliplatin (trans-1-diaminocyclohexane oxalateplatinum, Figure 1) is a third-generation platinum derivative that is widely used in the therapy of colorectal cancer. Compared with cisplatin, oxaliplatin has enhanced water solubility[65]. Research into the pharmacogenetics of oxaliplatin focused mainly on interindividual differences in oxaliplatin pharmacokinetics and genetic alterations in genes coding ABC/SLC transporters, DNA damage repair machinery, such as excision cross-complementing genes (ERCC1, ERCC2, OMIM: 126380, OMIM: 126340, respectively) and X-ray repair cross-complementing protein 1 (XRCC1, OMIM: 194360), and conjugating enzymes glutathione *S*-transferases (GSTM1, GSTP1, GSTT1, OMIM: 138350, OMIM: 134660, OMIM: 600436, respectively)[66] (Table 2).

The principal mechanism of action of oxaliplatin is inhibition of DNA synthesis in cancer cells by the formation of crosslinks in DNA. There is no evidence of cytochrome P450-mediated metabolism *in vitro*. Inactivation of reactive oxaliplatin species is mediated by conjugation to glutathione, which is mediated by GSTs. DNA damage by oxaliplatin is repaired mainly by nucleotide excision repair (NER), base excision repair (BER) and by replicative bypass[67]. Interestingly, the mismatch repair complex important for resistance to other platinum drugs has not been shown as crucial for oxaliplatin resistance[68].

Oxaliplatin is transported mainly by the uptake transporters SLC22A1/OCT1 (OMIM: 602607) and SLC22A2/OCT2 (OMIM: 602608), which play a critical role not only in cellular uptake but also in the associated cytotoxicity[69]. Other important transporters are human copper transporters (SLC31A1 and 2 OMIM: 603085 and 603088, respectively) that mediate cellular uptake of oxaliplatin and P-type ATPases ATP7A and 7B (OMIM: 300011 and 606882, respectively) that promote efflux of oxaliplatin and its sequestration into subcellular compartments[70,71].

ABCC2, ABCC4 and ABCC5 transporters are potentially involved in disposition of platinum compounds[72,73]. However, the data on clinical implications of the genotype and/or phenotype of these transporters with regard to platinum efficacy or toxicity are scarce and inconclusive[71].

GSTP1 is involved in the detoxification of cisplatin by the formation of cisplatin-glutathione adducts[74] and, consequently, the putative role of GSTs in resistance to platinum compounds is generally accepted. The suggested link between GSTs and the MAP kinase pathway may also contribute to the common mechanisms of resistance towards anticancer drugs[75].

Biomarkers of chemoresistance to oxaliplatin in colorectal cancer

Enhanced expression of members of ABCC family of efflux transporters can lead to a decreased cellular glutathione level and thus indirectly cause decrease of oxaliplatin inactivation[76]. Overexpression of ABCC2 and ABCG2 (OMIM: 603756) resulted in increased activity of oxaliplatin *in vitro*[77]. Oxaliplatin is administered in combination with 5-FU, and 5-FU significantly suppressed ATP7B and SLC22A2 and simultaneously increased ABCC2 mRNA expression[77]. The synergistic action of these two drugs on transport direction has been demonstrated *in vitro*. Low ATP7B mRNA and protein expression is associated with longer time-to-progression of colorectal cancer patients receiving oxaliplatin-based chemotherapy[78]. The role of ABCB1/P-glycoprotein in the oxaliplatin pathway has not yet been proven. Lack of association of ABCB1 transcript level in tumors with therapy outcome of colorectal cancer patients treated by FOLFOX has been reported recently[28]. However, ABCB1 polymorphisms were shown to be significantly associated with survival of colorectal cancer patients treated by oxaliplatin[79]. Carriers of the AG genotype in rs1045642 had significantly longer time to recurrence than AA homozygotes, and carriers of AA genotype in rs1128503 had better overall survival compared to GG homozygotes. Moreover, carriers of the AA-GG-TT haplotype constructed from rs1045642-rs1128503-rs2032582 polymorphisms had an inferior progression-free and overall survival compared to carriers of other haplotypes[79].

Excision nucleases such as ERCC1 and ERCC2 play a major role in the repair of DNA adducts in tumor cells after chemotherapy. Thus, in theory, low ERCC1 gene expression leading to a decreased DNA repair should be a positive predictive factor of therapeutic effect of oxaliplatin. High ERCC1 mRNA expression was associated with shorter overall survival in patients treated with oxaliplatin-based chemotherapy[80,81]. Overexpression of ERCC1 protein has been shown to represent an independent predictor of early failure of adjuvant therapy by FOLFOX and short disease-free and overall survival[80]. In contrast to the above-mentioned results, the prognostic importance of ERCC1 protein expression has not been observed in the phase Ⅲ CAIRO trial (n = 506)[49]. In another study, neither tumor ERCC2 nor XRCC1 protein expression was associated with overall survival of patients treated by adjuvant FOLFOX therapy[82].

Carriers of the GG genotype in rs11615 of *ERCC1* were reported to have better progression-free and overall survival than carriers of the A allele[83] after FOLFOX therapy, but this was not been confirmed in another study[84]. The latter study also found no association of rs13181 in another DNA repair gene *ERCC2* with the survival of colorectal patients treated by irinotecan. In an earlier study, Huang *et al*[85] observed a significant association of *ERCC2* polymorphism rs13181 with increased

Table 2 Putative biomarkers of efficacy and/or toxicity of oxaliplatin in colorectal cancer

Gene	Transcript	Protein	SNP	Effect	Ref.
ATP7B	Low	Low	-	Longer PFS	Martinez-Balibrea *et al*[78] 2009
ABCB1	No association	-	-	DFS and OS	Hlavata *et al*[28] 2012
	-	-	AG genotype in rs1045642	Longer DFS	Wu *et al*[79] 2013
	-	-	AA genotype in rs1128503	Longer OS	Wu *et al*[79] 2013
	-	-	AA-GG-TT haplotype in rs1045642-rs1128503-rs2032582	Shorter PFS and OS	Wu *et al*[79] 2013
ERCC1	High	-	-	Shorter OS	Shirota *et al*[80] 2001
	High	-	-	Shorter OS	Grimminger *et al*[81] 2011
	-	High	-	Shorter DFS and OS	Huang *et al*[82] 2013
	-	No association	-	PFS and OS	Koopman *et al*[49] 2009
	-	-	GG genotype in rs11615	Longer OS	Huang *et al*[83] 2011
	-	-	Rs11615	No association with OS	Martinez-Balibrea *et al*[84] 2011
ERCC2	-	No association		DFS and OS	Huang *et al*[110] 2013
	-	-	Variant allele in rs1052559	Shorter OS	Le Morvan *et al*[86] 2007
XRCC1	-	No association		DFS and OS	Huang *et al*[82] 2013
	-	-	CC genotype in rs25487	Longer OS	Huang *et al*[83] 2011
GSTM1 deletion	-	-	One copy	Longer OS	Funke *et al*[87] 2010
	-	-	Null genotype	Higher neutropenia	McLeod *et al*[90] 2010
GSTP1	-	-	AA genotype in rs1695	Inferior response	Kumamoto *et al*[88] 2013
	-	-	Rs1695	No association	Funke *et al*[87] 2010
	-	-	Rs1695	No association	Huang *et al*[85] 2008
	-	-	Rs1695	No association	Le Morvan *et al*[86] 2011
	-	-	AA genotype in rs1695	Higher neurotoxicity	McLeod *et al*[90] 2010
	-	-	Rs1695	No association with toxicity	Peng *et al*[91] 2013
GSTT1	-	-	Deletion	No association	Funke *et al*[87] 2010
	-	-	Deletion	No association	Huang *et al*[85] 2008
	-	-	Deletion	No association	Le Morvan *et al*[86] 2011

PFS: Progression-free survival; DFS: Disease free survival; OS: Overall survival.

risk of early relapse in patients with metastatic colorectal cancer treated by irinotecan-based chemotherapy. Carriers of the variant allele in *ERCC2* polymorphism rs1052559 had shorter disease-free and overall survival after treatment by oxaliplatin than wild type carriers[86].

XRCC1 is involved in repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents. Carriers of CC genotype in rs25487 of *XRCC1* had better progression-free and overall survival after treatment by FOLFOX4 than T allele carriers[83].

GSTM1 DNA copy number was inversely associated with survival in colorectal cancer patients treated with chemotherapy[87]. Mortality was significantly reduced in patients with one *GSTM1* copy (HR = 0.45, 95%CI: 0.23-0.90, P = 0.02) and non-significantly reduced in those with the *null* genotype (HR = 0.67, 95%CI: 0.35-1.27, P = 0.22) compared with carriers of two copies[87]. Neither functional *GSTP1* polymorphism rs1695 nor *GSTT1* deletion (*null*) were associated with survival of colorectal cancer patients in earlier studies[83,84,86,87]. However, a recent study reported that FOLFOX6-treated metastatic colorectal cancer patients (n = 63) with the *GSTP1*-rs1695 AA genotype had inferior responses to the treatment compared with G allele carriers[88].

Biomarkers of toxicity of oxaliplatin in colorectal cancer patients

A number of studies, including prospective clinical trials, provided conflicting results regarding the impact of polymorphisms in genes related to oxaliplatin mechanism of action and toxicity. No clear recommendations are currently ready for clinical practice. *ERCC1* rs11615 and *GSTP1* rs1695 are the most studied polymorphisms in relation to neutropenia. Carriers of the CC genotype in *XRCC1* rs25487 had decreased the risk of neuropathy in colorectal cancer patients treated by adjuvant FOLFOX[89]. Carriage of the AA genotype in GSTP1 rs1695 predisposed patients with the TT genotype treated by FOFOX in a large N9741 clinical study to increased neurotoxicity, but the meta-analysis provided by McLeod *et al*[90] and Peng *et al*[91] (2013) showed no association between GSTP1 polymorphism rs1695 and the development of neurotoxicity (Table 2).

IRINOTECAN

Irinotecan is a camptothecin analog (Figure 1). Irinotecan is converted to an active metabolite, SN-38, by carboxylesterases CES1 and CES2 (OMIM: 114835 and 605278, respectively)[92]. SLCO1B1 (OMIM: 604843) mediates the uptake of irinotecan in hepatocytes[93]. SN-38 is transported by ABC transporters, namely by ABCC1, ABCC2, ABCB1, and ABCG2[94-97]. Inside a cell, the active metabolite SN-38 inhibits topoisomerase I and subsequently stalls both DNA replication and transcription[98].

SN-38 is further metabolized by glucuronidation by uridine diphosphate glycosyltransferase 1A1 (UGT1A1, OMIM: 191740) to its inactive glucuronide conjugate, SN-38G[99]. An alternative pathway of irinotecan inactiva-

Table 3 Putative biomarkers of efficacy and/or toxicity of irinotecan in colorectal cancer

Gene	Transcript	Protein	SNP	Effect	Ref.
ABCB1	-	-	rs11288503	Decreased clearance of irinotecan	Mathijssen *et al*[103] 2003
ABCB1	-	-	rs112503T-rs2032582T-rs1045642T haplotype	Higher levels of SN-38	Sai *et al*[104] 2010
	-	-	rs112503T-rs2032582T-rs1045642T haplotype	Shorter OS	Glimelius *et al*[106] 2010
ABCG2	-	-	rs7699188	Associate with RR	De Mattia *et al*[105] 2013
	-	-	GG genotype in rs425215	Higher GI toxicity	Di Martino *et al*[117] 2011
ABCC2	-	-	CC genotype in rs717620	Longer PFS	Akiyama *et al*[109] 2012
	-	-	CC genotype in rs562	Higher GI toxicity	Di Martino *et al*[117] 2011
SLCO1B1	-	-	GA/AA genotype in rs2306283	Higher RR	Huang *et al*[110] 2013
	-	-	GA genotype in rs 2306283	Lower GI toxicity	Di Martino *et al*[117] 2011
SLC19A1	-	-	GG genotype in rs1051266	Higher RR	Huang *et al*[82] 2013
UGT1A1	-	-	28 allele in rs8175347[1]	No association with RR	Dias *et al*[115] 2012
	-	-	28 allele in rs8175347[1]	Lower RR	Marcuello *et al*[114] 2011
	-	-	28 allele in rs8175347[1]	Reduced activity of UGT1A1	Swen *et al*[62] 2011
	-	-	28 allele in rs8175347[1]	Increased toxicity, dose reduction recommended	Swen *et al*[62] 2011

[1]Due to lower tolerated dose. PFS: Progression-free survival; RR: Response rate; OS: Overall survival; GI: Gastrointestinal.

tion is oxidation, mediated by members of cytochrome P450 3A subfamily[100]. Research on the pharmacogenetics of irinotecan focused mainly on interindividual differences in genetic alterations of genes coding transmembrane transporters and irinotecan-metabolizing enzymes, such as UGT1A1[98] (Table 3).

Biomarkers of irinotecan chemoresistance in colorectal cancer

Deficiency of the Abcc4 protein in Abcb1a/b; Abcg2 (-/-) mice *in vivo* model significantly increased the brain concentration of all camptothecin analogs, suggesting a possible role of ABCC4 in irinotecan efflux[101]. Interestingly, 5-FU significantly decreased the expression of ABCC2, ABCB1, and ABCG2 in the small intestine and increased the concentration of SN-38 in the blood of rats[102]. Thus, a synergistic action on efflux transporters of these two drugs used in FOLFIRI regimen has been described in the rat *in vivo* model. Colorectal tumors had significantly downregulated ABCB1, ABCC4 and ABCG2 transcripts compared with non-malignant tissues from the same patients before any chemotherapy[28]. Therefore, it appears that colorectal tumors have favorable expression profiles of ABC transporters active in irinotecan efflux.

ABCB1 polymorphism rs1128503 has been associated with a decreased clearance of irinotecan in cancer patients (mostly with gastrointestinal malignancies)[103]. Higher SN-38 levels in carriers of the *2 haplotype, which harbors 1236C>T, 2677G>T and 3435C>T, in *ABCB1* (rs1128503T-rs2032582T-rs1045642T) among cancer patients receiving irinotecan have been observed[104]. On the other hand, *ABCB1* polymorphism rs2032582 was not associated with pharmacokinetic parameters of metastatic colorectal cancer patients receiving first-line FOLFIRI treatment[105]. The association of carrying the most common *ABCB1* haplotype (rs1128503T-rs2032582T-rs1045642T haplotype) with shorter overall survival was also reported[106]. The *ABCG2* rs2231142 T-allele reduced expression in comparison with the GG genotype and led to resistance towards irinotecan in cancer cell lines[107]. 15622C>T and rs7699188 variants of ABCG2 were associated with the response rate of colorectal cancer patients treated with FOLFIRI[105]. The haplotype ABCC2*2 (rs717620C-rs2273697A-rs3740066C) was associated with lower irinotecan clearance in Caucasian cancer patients[108]. Akiyama *et al*[109] analyzed *ABCC2* polymorphisms in a well-defined cohort of Japanese colorectal cancer patients who harbored *UGTA1*1/*1*, **1/*6*, or **1/*28* genotypes, which are associated with similar irinotecan pharmacokinetics and responses to FOLFIRI. The *ABCC2* rs717620 CC genotype has been associated with a higher response rate and longer progression-free survival, followed by CT and TT genotypes[109].

Genotype GA/AA of SNP rs2306283 of the gene SLCO1B1 and genotype GG of SNP rs1051266 of the gene SLC19A1 were associated with a higher response rate in colorectal cancer patients treated with irinotecan[110].

The expression of CES1 and CES2 is organ-specific. CES1 is expressed mainly in the liver, whereas CES2 is predominantly expressed in the small intestine. CES1 is 100-fold less effective than CES2 at drug activation of irinotecan, but plays a significant role, mainly in the liver[111]. Until recently, CES was believed to play only a minor role in irinotecan metabolism and no functional polymorphisms have been identified[112]. Sai *et al*[104] reported a gene-dosage effect of functional *CES1A* on SN-38 formation for the first time and suggested its potential role in irinotecan toxicity.

The reduced mRNA expression of UGT1A1, 1A3, 1A4, lA6, 1A9 and overexpression of UGT1A5, UGTlA8 and UGTlAl0 in colorectal tumors compared with non-malignant tissues from the same patient has recently been reported[113]. Interestingly, despite the fact that UGT1A1 is the most studied gene with regard to irinotecan toxicity, little is known about the association of its expression

with the prognosis of colorectal cancer patients. Tailored dosing based on UGT1A1 genotype was tested in a prospective trial[114]. The dose of irinotecan in the biweekly schedule (standard dose 180 mg/m^2) could be escalated to 450 mg/m^2 in patients with the UGT1A1*1/*1 genotype and to 390 mg/m^2 in patients with the UGT1A1*1/*28 genotype, but only to 150 mg/m^2 in patients with the UGT1A1*28/*28 genotype. The maximum tolerated dose was 390 mg/m^2, 340 mg/m^2 and 130 mg/m^2 for patients with the UGT1A1*1/*1, UGT1A1*1/*28 and UGT1A1*28/*28 genotypes, respectively. Significantly higher objective response rates was observed in patients with the UGT1A1*1/*1 (60%) and UGT1A1*1/*28 (39%) genotypes compared with patients with the UGT1A1*28/*28 genotype (13%). A marked difference in response rate was observed between patients treated with an irinotecan dose of 260 mg/m^2 and higher (67%) *vs* patients treated by the lower dose (24%). In the multivariable logistic regression analysis irinotecan 260 mg/m^2 or higher was the only predictor of objective response. The time-to-disease progression was also significantly increased in patients treated with higher irinotecan dose[114].

According to the meta-analysis published by Dias *et al*[115], the individual response to irinotecan is unlikely to be affected by the presence of the *UGT1A1*28* variant because of the lack of statistically significant differences in objective response rates between irinotecan-administered cancer patients divided by *UGT1A1* polymorphism rs8175347.

Biomarkers of toxicity in colorectal cancer patients treated with irinotecan

Studies on polymorphisms in ABC transporters have shown some promising results; several potential biomarkers have been describe such as *ABCB1* (rs1045642), *ABCC2* (rs3740066), *ABCC5* (rs562), *ABCG1* (rs425215) and *ABCG2* (rs3832043) (Table 3)[106,112,116,117]. However, in terms of clinical practice, The Royal Dutch Pharmacists Association (RDPA) posted the only recommendation on genetic testing of colorectal cancer patients before treatment by irinotecan in FOLFIRI. The Pharmacogenetics Working Group of RDPA has assessed therapeutic dose recommendations for irinotecan pursuant to the *UGT1A1* genotype. Dose reduction for *UGT1A1*28* homozygous patients receiving more than 250 mg/m^2 has been recommended[62]. This recommendation is not applicable for FOLFIRI regimen where a dose of 180 mg/m^2 is prescribed. Homozygous carriers for the *UGT1A1*28* allele have reduced UGT1A1 enzyme activity leading to an increased risk for neutropenia[62].

RESULTS OF CLINICAL TRIALS ON BIOMARKERS FOR FOLFOX/FOLFIRI REGIMENS

Studies on predictive biomarkers useful for deciding whether patients should be treated by FOLFOX or FOLFIRI are currently lacking. Only a few studies on colorectal carcinoma patients treated by regimens involving FOLFOX may be found in the recent literature. Watanabe *et al*[118] analyzed microarray expression profiles of 46 patients with metastatic or recurrent colorectal carcinoma who received modified FOLFOX6. As a result, a 27-gene FOLFOX response predictor with an overall accuracy of 92.5% was constructed for selection of patients who may benefit from therapy with this regimen in the adjuvant or advanced disease setting[118]. Another Japanese study suggested that the absence of ERCC1 or GSTP1, but not TYMS, as assessed by immunohistochemistry, predicts a favorable response of colorectal cancer patients to FOLFOX[119]. Interestingly, concurrent methylation of transcription factor neurogenin 1 (NEUROG1, OMIM: 601726) and tumor suppressor p16 (CDKN2A, OMIM: 600160) was recently associated with shorter DFS following adjuvant FOLFOX in Stages Ⅱ/Ⅲ colorectal cancer[120].

Carriers of the T allele in *ERCC1* polymorphism rs11615 among colorectal cancer patients (n = 168) had significantly lower response to FOLFOX and shorter PFS than CC wild-type genotype carriers[121]. Similarly, FOLFOX4-treated colorectal cancer patients with Gln allele in *ERCC2* rs13161 had a significantly shorter PFS and OS than Lys/Lys wild-type carriers[122]. The same authors also reported that Val allele (rs1695 in GSTP1) carriers had longer PFS and OS, but also a significantly higher incidence of grade 3/4 cumulative neuropathy after FOLFOX4 therapy than wild-type Ile/Ile carriers[123]. Metastatic colorectal cancer patients treated with FOLFOX4 carrying either the CC genotype in *ERCC1* rs11615 or the GG genotype in *XRCC1* polymorphism rs25487 had significantly longer PFS and OS than the respective variant allele carriers[83]. The combination of these two polymorphisms indicated an even higher survival benefit than carriage of a single predictor genotype[83].

There are currently no studies focusing on the evaluation of predictive biomarkers of FOLFIRI in colorectal cancer patients. Therefore, a retrospective study based on putative predictive biomarkers for 5-FU and irinotecan so far identified (Tables 1 and 3) are now highly desirable in a cohort of patients treated with this regimen.

Future directions

Despite remarkable progress during the past two decades that resulted from the introduction of new drugs, including targeted agents, the potential of systemic pharmacologic therapy has still not been fully translated into effective treatments in daily practice. Optimized algorithms for use of the available active agents are urgently needed. The pharmacogenomic approach has been promising in not only identifying the patients at increased risk of toxicity, but also in allowing a more tailored approach in pharmacotherapy of colorectal carcinoma.

From the reviewed information it is obvious that there are many important factors in resistance of colorectal tumor cells to FOLFOX/FOLFIRI regimens. The broad individual variability and cell- and tissue-specific

pattern of expression, including complicated regulation, cause difficulties in drawing conclusive remarks on clinical application of currently identified putative biomarkers. The lack of functional studies on relations between genotype and phenotype, including activity and pharmacokinetics, of the majority of biomarkers also greatly complicates translation of results into biologically-relevant mechanisms that are necessary for recommendations on the further use of biomarkers.

The majority of studies reviewed provided conflicting results, which can be attributed to the different methodologies used, such as antibodies for immunohistochemistry, lack of statistical power, possible selection bias associated with the heterogeneity of the cohorts of patients in terms of ethnicity or clinical characteristics, including treatment, drug-drug interactions as well as drug-alimentary interactions.

The unprecedented increase in the knowledge concerning the pathogenesis of malignant tumors, together with the advent of new agents, has created a basis for clinical cancer pharmacogenetics. Enormous progress in the area of analysis of both genotype and phenotype that has been made possible by the advent of next generation sequencing, the application of strict methodical guidelines such as MIQE or REMARK, and the establishment of public databases and pharmacogenetics consortia for rigorous evaluation of existing studies may be instrumental for further advancement of our knowledge in this field.

The pursuit of research in the following directions may further accelerate the evolution of the field of pharmacogenetics: (1) studies on the functionality of haplotypes and the associations with cellular and clinical phenotypes should shed more light on the utility of the biomarkers identified so far. Such exploration is now made much easier with the advent of next generation sequencing; (2) very little is known about the regulation of expression and posttranslational processing of putative biomarkers. Also the role of epigenetic factors, such as methylation of promoter regions or noncoding RNAs will require more investigation. Interaction of drug transporters or metabolizing enzymes with oncogenic or tumor suppressor pathways is also a highly attractive topic because there are already several examples of such interactions, including p53, N-myc, or ERK/MAPK, but their clinical relevance remains unknown; and (3) the time for prospective clinical trials aimed at personalized chemotherapy defined by biomarkers has already come. Genotyping is not routinely used for prediction of therapeutic effect or toxicity of anticancer drugs in clinical practice. The lack of alternative treatments for patients carrying the risk genotype represents the main obstacle. It is also essential to clearly determine the sensitivity and specificity of genotyping tests and to define positive and negative predictive values before the introduction of these methods into routine practice.

Taken together, there are many opportunities to increase our insight into the importance of the above reviewed putative biomarkers of drug resistance and to look for others. The final profile of the drug-sensitive or resistant patient will most probably be a mixture of genetic, epigenetic, intracellular and extracellular factors, whose timing and interaction with extrinsic factors should also be considered.

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