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Determinants of chemosensitivity in gastric cancer

David J Park and Heinz-Josef Lenz

Recent advances in the management of gastric cancer, especially in the arena of chemotherapy, are paving the way for optimization of treatment that maximizes effectiveness while minimizing toxicity. The expansion of the chemotherapeutic armamentarium has led to multiple combinations of cytotoxic agents. Unfortunately, the benefit of chemotherapy has been modest at best, and no one combination has shown significant superiority over the others in comparative Phase III trials. It is in this setting that pharmacogenetic advances have the potential to play an important role in achieving superior clinical outcome among different subsets of patients through prospective prediction of clinical benefit to particular regimens. We are just beginning to make inroads in gastric cancer pharmacogenetics, mostly through small, pilot retrospective studies. Several potential candidates, such as thymidylate synthase, excision repair complementation group 1 and glutahione S-transferase P1, have been identified so far and more are bound to surface, especially when biologic therapies are added to the armamentarium. Serious challenges lay ahead given the complex nature of cytotoxic metabolism with multiple players working together to influence drug effectiveness and/or toxicity. Well-designed large prospective trials are needed to identify key genes among the multiple potential candidates that can help a clinician make real-time treatment decisions in respect to a particular regimen depending on a patient's pharmacogenetic profile.

Addresses
Division of Medical Oncology, University of Southern California/Norris Comprehensive Cancer Center, Keck School of Medicine, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA

Corresponding author: Lenz, Heinz-Josef (lenz@hsc.usc.edu)

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Introduction

Gastric cancer is the forth most common type of cancer and the second leading cause of cancer-related death in the world [1]. Surgery continues to have an essential role in the management of this disease, potentially leading to long-term survival and even cure, although the extent of surgery continues to be a subject of debate. A closely linked issue is the role of neo and adjuvant therapy, which is still being defined. However, at least in the USA, post-operative chemoradiotherapy has become standard of care in the treatment of localized gastric cancer [2].

Unfortunately, early gastric cancer rarely presents with noticeable symptoms. Consequently, a significant number of patients are diagnosed with advanced disease where the five-year survival rate is <5%. Moreover, significant relapse rates, even after curative surgery and adjuvant therapy, remain a challenging problem.

Chemotherapy appears to be a useful tool in the management of advanced gastric cancer, with several randomized trials demonstrating a modest but real benefit in prolongation of survival and maintenance of quality of life.

Various agents and their combinations were shown to be effective. 5-Fluorouracil (5-FU) is the earliest and yet one of the most important cytotoxic agents in the management of this disease. Cisplatin, which is often used in combination with 5-FU, has also shown activity. In fact, the combination of 5-FU and cisplatin is regarded as a standard chemotherapy. Additional agents have been introduced recently with promising efficacy. These include the oral fluorpyrimidines, taxanes, irinotecan and the new platinum analog oxaliplatin.

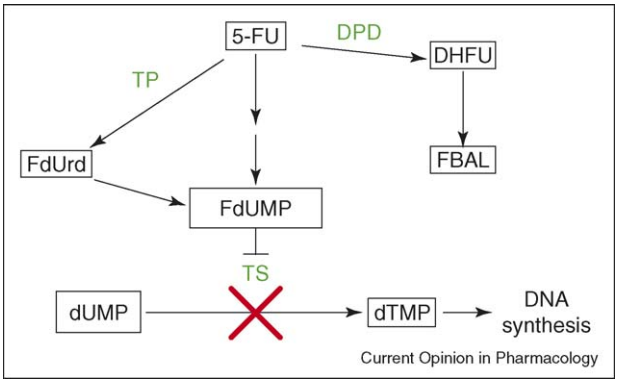
This review focuses on current developments pertaining to the area of pharmacogenomics and chemosensitivity in the treatment of gastric cancer, principally in the advanced stage. In particular, we will discuss four agents/classes that are most commonly utilized: fluoropyrimidines, platinums, irinotecan and taxanes. For a more comprehensive overview of gastric cancer and its treatment the reader is referred to recent outstanding reviews [3•,4•].

Fluoropyrimidines

Since its introduction in 1957, 5-FU has remained an integral player in the management of gastrointestinal malignancies, including gastric cancer. 5-FU is converted into its active metabolite 5-fluoro-2-deoxyuridine (Figure 1). Its main mechanism of action is the inhibition of thymidylate synthase (TS) through the formation of a stable ternary complex. This prevents the only *de novo* source of thymidine, which is essential in DNA synthesis. Intracellular 5-FU levels are also influenced by thymidylate phosphorylase, dUTPase and dihydropyrimidine dehydrogenase (DPD).

5-FU-based therapy (especially combined with cisplatin) is considered standard therapy in the treatment of

Figure 1



5-FU pathway. 5-FU is converted into 5-fluoro-deoxyuridine monophosphate (FdUMP), an irreversible inhibitor of thymidylate synthase (TS). This prevents the formation of thymidine 5′-monophosphate (dTMP), thus inhibiting DNA synthesis. Dihydropyrimidine dehydrogenase (DPD) is responsible for degradation of 5-FU into its inactive metabolite 5,6-dihydro-5-FU (DHFU), and then α-fluoro-β-alanine (FBAL). Thymidine phosphorylase (TP) mediates the conversion of 5-FU into its derivative 5-fluoro-2′-deoxyuridine (FdUrd).

advanced gastric cancer and a reference arm in most clinical trials. 5-FU–cisplatin combinations have shown response rates ranging from 20 to 50% [4•]. Its main toxicities are mucositis, neutropenia, gastrointestinal toxicities and, if combined with cisplatin, neuropathy and renal toxicities can also be observed.

Newer fluoropyrimdines such as capecitabine and S-1 (both oral agents) are undergoing active investigation in combination with other cytotoxic agents. Several Phase II trials have demonstrated similar response rates to 5-FU-based therapies with differing dose-limiting toxicities.

Thymidylate synthase

As previously stated, a key enzyme inhibited by 5-FU is TS. Gene expression levels of *TS* measured either as mRNA [5] or protein production [6] were shown to be associated with clinical outcome to 5-FU-based chemotherapy in gastric cancer. Higher levels of *TS* were associated with lower response rates to 5-FU-based chemotherapy as well as shorter survival.

Regulation of *TS* expression is an area of active research. Various studies have shown that a polymorphic tandem 28-bp repeat in the promoter region of the *TS* gene possesses a regulatory role and influence its expression [7,8]. An allele of this *TS* enhancer region (TSER) polymorphism usually contains double or triple tandem repeats leading to the genotypes 2R/2R, 2R/3R and 3R/3R. Although rare, alleles with 4 and 5 repeats have also been described [9]. Moreover, there seems to be inter-ethnic variability in the frequency of 2R or 3R alleles [10].

The 2R allele has been shown to be associated with lower gene expression compared with the 3R allele. It has been proposed that the effect of this tandem repeat might be on the transcriptional and/or translational efficiency of the *TS* gene [7].

The potential usefulness of TSER polymorphism in the prediction of clinical outcome of patients with gastrointestinal malignancies treated with fluoropyrimidine therapy is a subject of intense investigation. The hypothesis is that patients carrying the 3R allele associated with higher *TS* activity and expression would fare worse under fluoropyrimidine-based therapy. This has been shown in various studies in both metastatic and locally advanced colorectal cancer [11–14]. There is even some preliminary evidence that TSER polymorphism might be associated with response to oral capecitabine [15].

Recent studies are shedding light into the role of TSER polymorphism in patients with gastric cancer treated with fluoropyrimidines. Ishida *et al.* [16] examined the association of TSER polymorphism with *TS* gene expression and prognosis in patients with gastric cancer. Patients homozygous for the 3R genotype were found to be more advanced in stage and have shorter survival, although its impact was less clear in patients treated with oral fluoropyrimidines [16].

Interestingly, a simple assessment of the TSER repeat polymorphisms might not adequately harness the predictive power of *TS*. A novel G → C single nucleotide polymorphism (SNP) found at a relatively high frequency has been identified in the second repeat of the 3R allele, which is a polymorphism within a polymorphism. This polymorphic change abolishes upstream transcription factor 1 (USF-1) binding and alters transcriptional activation of *TS* [17••,18•] In addition, a 6-bp polymorphic deletion in the 3′ untranslated region (3′-UTR) of *TS* has been shown to be associated with decreased mRNA stability and to be in linkage disequilibrium with TSER repeat polymorphism [19•].

Recently, a retrospective study assessing various polymorphisms of key genes in 52 patients with advanced gastric cancer treated with 5-FU–cisplatin chemotherapy was reported. Analysis of gene polymorphisms involved in the fluoropyrimidine pathway was carried out taking in consideration the novel G → C SNP within TSER. The favorable (low *TS* expression) TSER genotype group included 2R/2R, 2R/3RC and 3RC/3RC; and the unfavorable (high *TS* expression) TSER genotype group included 2R/3RG, 3RC/3RG and 3RG/3RG. The favorable group showed a trend for improved survival (10.2 versus 6.0 months, $p = 0.099$) compared with the unfavorable group [20••].

Another study evaluated the role of *TS* 3′-UTR polymorphism in 106 patients with advanced gastric cancer

treated with 5-FU-based chemotherapy. The authors found that patients with both alleles containing the 6-bp nucleotide fragment ($n = 8$) did not respond to 5-FU-based chemotherapy compared with patients with at least one allele without the 6-bp nucleotide fragment [21•].

Finally, Kawakami *et al.* [22] investigated the prognostic role of *TS* polymorphisms in 90 patients with gastric cancer treated with radical surgery and adjuvant 5-FU-based chemotherapy. They combined the TSER (or 5′-UTR) and the 3′-UTR polymorphisms taking into account TSER repeats, G → C SNP and the 3′-UTR 6-bp deletion. Patients were distributed into four groups of *TS* expression: 5′-UTR*low*/3′-UTR*low* (25 patients), 5′-UTR*low*/3′-UTR*high* (19 patients), 5′-UTR*high*/3′-UTR*low* (36 patients) and 5′-UTR*high*/3′-UTR*high* (10 patients). 'Low producers of TS', which are patients with 5′-UTR*low*/3′-UTR*low*, had the best clinical outcome in terms of disease-free and overall survival, especially when compared with 5′-UTR*high*/3′-UTR*low* or 5′-UTR*high*/3′-UTR*high* [22].

Dihydropyrimidine dehydrogenase

5-FU catabolism is principally carried out by DPD, which is responsible for the degradation of 80–90% of 5-FU to its inactive metabolite 5,6-dihydro-5-FU [23]. Significant interindividual variability is seen in DPD activity [24,25]. Low levels of DPD have been shown to be associated with 5-FU-induced toxicity (gastrointestinal, hematologic, neurological), including death in some patients [26–29]. The activity of mutated DPD is severely compromised, especially in individuals homozygous for a common G → A point mutation in the invariant GT splice donor site flanking exon 14 (IVS14 + 1G>A) leading to loss of exon 14 and a truncated protein. Its frequency is 0.91% in the Caucasian population, and patients with this mutation were shown to be at a higher risk for severe toxicities [30].

Research on the role of DPD as a predictor of 5-FU sensitivity in gastric and other neoplams is still in its early stages. Retrospective trials, mostly with small number of patients, have shown weak to moderate correlation between low activity and expression of DPD, and improved outcome to 5-FU-based chemotherapy [31–34].

Irinotecan

Irinotecan (CPT-11) is a water-soluble chemical derivative of camptothecin. As an inhibitor of topoisomerase I, CPT-11 interferes with DNA replication leading to double-strand DNA breaks and cellular death. CPT-11 is active in a wide array of malignancies including gastric cancer. Combination regimens with CPT-11 have shown to achieve response rates of 35–50% [4•]. The liver is the main site where CPT-11 is converted into its active metabolite SN-38 (Figure 2). This conversion is mediated by human liver carboxyesterase (CES). Oxidative metabolism of CPT-11 and SN-38 is accomplished through the cytochrome P450 isoforms 3A4 and 3A5 (CYP3A4 and CYP3A5). Uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) glucoronidates SN-38 to its inactive metabolite SN-38G. Moreover, elimination of CPT-11 through efflux transport is mediated by ABCB1 (multidrug resistance protein 1) and ABCB2 (multidrug resistance protein 2) [35].

Many other proteins in addition to the ones mentioned above (CES, CYP3A4 and 3A5, UGT1A1, ABCB1 and ABCB2) are involved in the irinotecan pathway. Recently, a gene profiling study of 24 genes involved in the irinotecan pathway in patients with colorectal cancer revealed differences in expression of several genes in tumor and normal tissues. Using hierarchical clustering, investigators were able to derive three distinct patient groups that had significant differences in RNA expression level of seven genes [36]. These could be preliminary steps in the identification of genes or a gene profile that might be able to predict sensitivity to irinotecan.

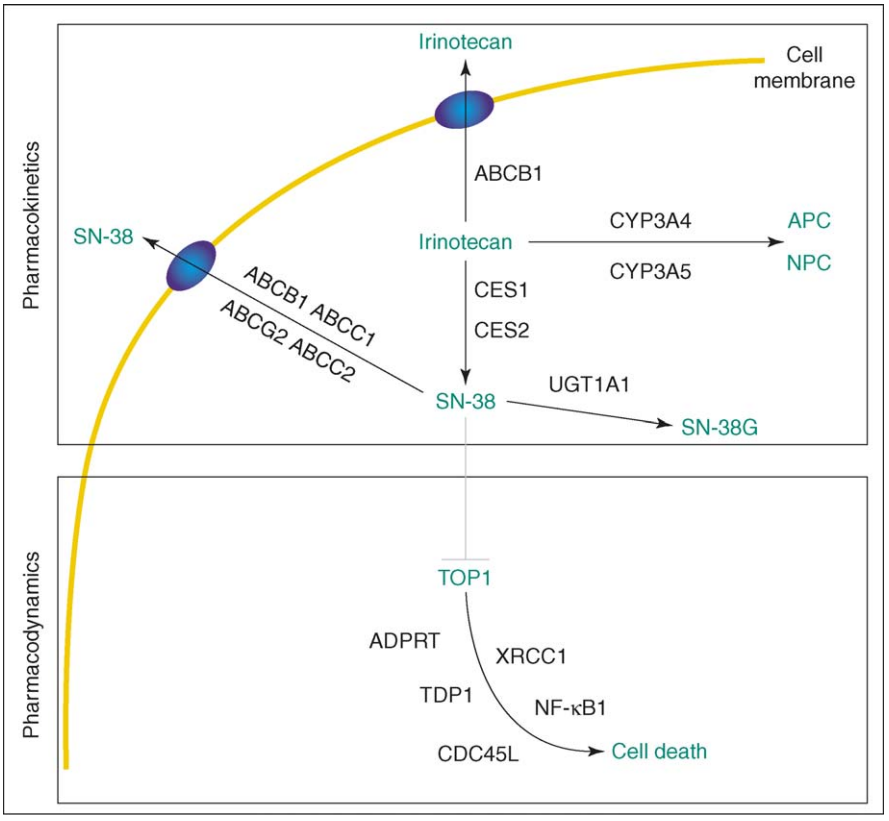
A study attempting to link genetic polymorphisms in transporters and enzymes involved in irinotecan elimination with differential interindividual drug exposure was recently published. Eighteen genetic variants in nine genes involved in irinotecan pathway were tested in 65 patients with cancer receiving irinotecan. The authors found that the homozygous T allele of the efflux transport *ABCB1* 1236C → T polymorphism (a 'silent' polymorphism in codon 411) was associated with higher exposure to irinotecan and SN-38. The other variants tested showed no such correlation, which could be due to low allele frequency [37]. These preliminary data highlight not only the potential but also the challenges of identifying clinically useful genes or gene profiles for chemosensivity in a drug with such a complex metabolism as irinotecan.

Uridine diphosphate glucuronosyltransferase 1A1

Interestingly, the most significant progress made in irinotecan pharmacogenomics is in predicting toxicity. As stated before, the hepatic isoform 1A1 of UGT is responsible for the glucuronidation and detoxification of SN-38 to its inactive SN-38 glucoronide [38]. A common polymorphism of the *UGT1A1* gene leads to an additional TA repeat in the TATA sequence of the *UGT1A1* promoter, with the longer repeat (seven versus six) associated with a significant decrease of SN-38 glucoronidation, potentially leading to increased toxicity. Up to 33% of Caucausian carry the variant allele with seven repeats [39].

The ability of the *UGT1A1* polymorphism to predict severe gastrointestinal and bone marrow toxicity was demonstrated in an earlier study with a small number of patients ($n = 20$) with solid tumors treated with irinotecan. Patients with the seven TA repeat allele (either 6/7 or 7/7) were more likely to experience severe grades of neutropenia and diarrhea [40].

Figure 2



Irinotecan pathway. Irinotecan is converted by carboxyesterase (CES) to its active metabolite SN-38. SN-38 binds to and stabilizes the topoisomerase I (TOP1)–DNA complex preventing religation of DNA, thus leading to cell death. SN-38 undergoes glucuronidation into its inactive metabolite SN-38G by uridine diphosphate glucuronosyltransferase (UGT1A1). It is also transported outside the cell by efflux proteins ATP-binding cassette (ABC). Cytochrome 450 (CYP) is also involved in the metabolism of irinotecan. Abbreviations: ADPRT, ADP-ribosyltransferase; APC, 7-ethyl-10[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin; CDC45L, CDC45 cell division cycle 45-like; NFκB, nuclear factor kappa B; NPC, 7-ethyl-10[4amino-1-piperidino]-carbonyloxycamphothecin; TDP, tyrosyl-DNA phosphodiesterase; XRCC1, X-ray repair complementing defective repair in Chinese hamster cells. Figure reproduced with permission of Future Medicine Ltd [67].

Most recently, large-scale prospective pharmacogenetic data from patients with metastatic colorectal cancer treated with irinotecan-based chemotherapy (North Central Cancer Treatment Group N9741) demonstrated that those homozygous for the variant allele (seven repeats) had significantly higher rates of grade 4 neutropenia (36%) compared with those with the more common variant (8.6%) [41•]. This has led to an update of irinotecan's package insert, which warns that patients with the variant *UGT1A1* polymorphism might be at a higher risk for severe neutropenia (www.pfizer.com). Unfortunately, dose adjustment recommendations as a function of *UGT1A1* polymorphisms are lacking thus far.

Platinums

Platinum agents have shown effectiveness in the treatment of gastric cancer. Both cisplatin and its third-generation analogue oxaliplatin-based therapies have significant clinical benefit [4•]. Their mechanism for cytotoxicity is thought to be through DNA alkylation and formation of DNA adducts that result in inhibition of DNA synthesis, function and transcription. Several mechanisms of resistance to platinum compounds have been identified. These include decrease drug accumulation, caused by alteration in cellular transport, drug inactivation by sulfhydryl-containing proteins, such as glutathione, and enhanced DNA repair [42]. One of the major DNA repair systems in mammalian cells is the nucleotide excision repair (NER) pathway. In fact, NER is the only known mechanism in mammalian cells for the removal of bulky, helix-distorting DNA adducts produced by platinum agents [43].

Excision repair complementation group 1

Excision repair complementation group 1 (ERCC1) is a highly conserved protein and an essential member of the NER pathway [44]. The ERCC1–XPF (xeroderma pigmentosum group F) complex is involved in the cleavage of damaged DNA strand 5′ to the DNA lesion. Several studies have shown an association between *ERCC1*

expression and clinical outcome to platinum-based chemotherapy, including gastrointestinal malignancies [45–47]. In an earlier retrospective study, intratumoral *ERCC1* mRNA levels in patients with advanced gastric cancer treated with 5-FU and cisplatin were correlated with response and overall survival. In patients with both low *TS* and *ERCC1* mRNA levels, the response rate was 85%, whereas in those with high *TS* and *ERCC1* mRNA levels, the response rate was 20% [45].

Two common polymorphisms of the *ERCC1* gene have been identified. The first SNP at codon 118 causes a C → T change coding for the same amino acid, asparagine, and could affect codon usage. An earlier analysis by Park *et al.* [48], on a small number of patients ($n = 31$) showed that as the number of T alleles increased a trend toward higher intratumoral *ERCC1* mRNA was seen. The second *ERCC1* SNP causes a C → A change and is located in position 8092 in the 3′-UTR. The role of these polymorphisms in platinum sensitivity and toxicity has been studied retrospectively in various neoplasms, including melanoma [49], lung [50–53] and colon [54–56] cancers with variable and often contradictory results. Differences in populations, tumor types, therapeutic regimens and assessment of clinical outcome might have led to diverging results. Nevertheless, these studies provide compelling evidence that *ERCC1* polymorphisms might be important in platinum sensitivity and toxicity and should be further studied in large, well-designed prospective clinical trials.

Xeroderma pigmentosum group D

Another vital member of the NER is the xeroderma pigmentosum group D (*XPD*) gene, also known as *ERCC2*. It has a central role in the recognition of damaged DNA along with other proteins. Although an earlier study in a small number of ovarian cancer patients did not show a correlation between *XPD* gene expression and cisplatin resistance [46], a more recent study showed a significant correlation between XPD protein levels and resistance to alkylating agents [57].

Two common genomic polymorphisms in the *XPD* gene with potential functional significance have been identified. One single nucleotide A → C polymorphism leads to Lys → Gln change in codon 751. The second is located in exon 10 and is a G → A change that leads to a Asp312Asn variation. The role of these polymorphisms as predictors of platinum sensitivity has been studied in a variety of neoplasms including gastric cancer, with variable findings. In colorectal cancer, XPD Lys751Gln was found to be correlated with response and overall survival in a retrospective study of 73 patient treated with oxaliplatin-based chemotherapy [58]. A large prospective pharmacogenetic adjunct study (N9741) confirmed differential response rates to oxaliplatin-based therapy according to the XPD751 polymorphism [41•]. In patients with lung cancer treated with cisplatin-based chemotherapy, XPD Asp312Asn was correlated with overall survival. However, neither XPD751 nor XPD312 polymorphisms had predictive activity in other studies involving patients treated with platinum-based chemotherapy [52,53]. Lastly, a small retrospective study investigating the predictive value of multiple gene polymorphisms in patients with advanced gastric cancer did not show a correlation between XPD Lys751Gln polymorphism and clinical outcome to cisplatin-based chemotherapy [20••].

Glutahione S-transferase P1

Phase II detoxification enzymes such as the glutathione-S-transferases (GSTs) are also thought to be involved in platinum resistance. The glutahione S-transferase P1 (*GSTP1*) isoform is overexpressed in gastrointestinal malignancies, and *in vitro* studies have demonstrated its significant participation into detoxification and, thus, resistance to platinum agents [59]. A SNP substitution of A → C, causing an Ile → Val change at codon 105 has been associated with a significant decrease in enzymatic activity. Patients with the valine allele were shown to have superior clinical outcome to oxaliplatin-based chemotherapy for colorectal cancer [60].

A recent study has examined the role of *GST* (*P1*, *T1*, *M1*) polymorphisms as well as polymorphisms in four other genes as clinical predictors of 5-FU–cisplatin chemotherapy in advanced gastric cancer. This retrospective study of 52 patients showed that patients homozygous for the *GSTP1* valine allele had a significantly higher response rate (67% versus 21%) and median survival (15 versus 6 months) [20••]. Interestingly, a similar pattern was seen in patients with Hodgkin's lymphoma [61] and breast cancer [62] treated with cytotoxic chemotherapy. It should be noted that in the N9741 pharmacogenetic study *GSTP1* polymorphisms were not significantly associate with response rates in oxaliplatin-based therapy [41•].

Taxanes

Taxanes, namely paclitaxel and docetaxel, have demonstrated promising activity in gastric cancer. Response rates of single agent taxanes range between 10–25% and 20–50% when used in combination with other cytotoxics. Metabolism of taxanes through hydroxylation is mediated by isoforms of cytochrome P450 (CYP2C8, CYP3A4 and CYP3A5). In addition, efflux protein ABCB1 might influence taxane efficacy via hepatobiliary or intestinal secretion [63].

An earlier study had shown that polymorphic variants within CYP2C8 might influence taxane metabolism with potential impact on efficacy [64]. However, a more recent study investigating the association between polymorphisms in CYP2C8, CYP3A4, CYP3A5 and ABCB1, and pharmacokinetics of paclitaxel in a cohort of 97 Caucasians failed to show any significant relationship [65].

Conclusion

The chemotherapeutic armamentarium has seen significant expansion in recent years leading to various synergistic combinations. Unfortunately, effectiveness across the differing combinations is modest, with no one combination showing clear superiority. The addition of new agents including biologics (eg. monoclonal antibodies) could significantly improve clinical outcome. Advances in pharmacogenetics might also lead to improvements of clinical outcome by prospectively identifying particular subsets of patients that could benefit the most from particular cytotoxic combinations. To date, relatively small retrospective studies have identified TS, ERCC1 and GSTP1 as potential candidates. The complex nature of cytotoxic metabolism, which often involves multiple players, the variable frequencies of genetic polymorphisms and the relatively small number of patients in the published studies pose significant limitations in our current knowledge of gastric cancer pharmacogenetics. Larger well-designed studies are needed to confirm the above findings, as well as the investigation of other potential candidates that might not have shown significant associations with clinical outcome in the aforementioned early studies. We expect future research to yield important clues that might help guide a clinician make real-time decisions to treat a patient with a particular regimen.

Update

Since the writing of the current review, a recent study on gastric pharmacogenetics has been published. Ruzzo *et al.* [66] have studied prospectively the predictive value of a panel of putative gene polymorphisms for fluorouracil–cisplatin chemotherapy in advanced gastric cancer. A relatively large number of patients ($n = 175$) were enrolled. Thirteen polymorphisms in nine genes were assessed. Chemoresistance and poor survival were significantly associated with *TS* 5′-UTR 3G-genotype (2R/3G, 3C/3G, 3G/3G) and *GSTP1* 105 A/A homozygous genotype. This study confirms earlier findings on a smaller, retrospective trial [20••].

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