Pharmacogenetics of capecitabine and oxaliplatin in treatment of advanced colorectal cancer

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Pharmacogenetics in chemotherapy of colorectal cancer

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Abstract

Although in recent years, chemotherapeutic options for colorectal carcinoma have expanded, overall response rates are still too low, with high rates of toxicity. Pharmacogenetics aim at predicting both treatment response and adverse effects in individual patients.

This review describes the current knowledge of pharmacogenetic markers in the systemic treatment of colorectal cancer. *UGT1A1*28* leads to reduced conjugation of SN-38, the active metabolite of irinotecan, resulting in an increased rate of adverse effects, especially neutropenia. To a lesser extent, increased 5-FU toxicity is predicted by *DPYD*2A*. A variable number of tandem repeats polymorphism in the thymidylate synthase enhancer region, in combination with a single nucleotide polymorphism C>G, may predict poorer response to 5-FU. Efficacy of oxaliplatin is influenced by polymorphisms in components of DNA repair systems, such as *ERCC1* and *XRCC1*. Polymorphic changes in the endothelial growth factor receptor probably predict cetuximab efficacy. Furthermore, the antibody-depended cell-mediated cytotoxic effect of cetuximab may be reduced by polymorphisms in the immunoglobin G fragment C receptors. Bevacizumab efficacy is suspected to be influenced by polymorphisms in the *VEGF* gene and the hypoxia inducible factor 1α gene. Although the interpretation of pharmacogenetic studies is complicated, results imply a promising way of pretreatment prediction of chemotherapy efficacy and toxicity.

Introduction

In the last decades, important developments in the chemotherapeutic treatment of colorectal cancers have taken place. Since its discovery in 1957, 5-fluorouracil (5-FU) has played an important role in the therapy of colorectal carcinoma, both in adjuvant treatment combined with oxaliplatin, as well as in metastatic disease, where it is also combined with irinotecan. Recently, the anti-vascular endothelial growth factor (anti-VEGF) monoclonal antibody bevacizumab and the human epidermal growth factor receptor (EGFR)-targeted monoclonal antibodies cetuximab and panitimumab have been included in the treatment for advanced colorectal carcinoma. The addition of either of these compounds to conventional chemotherapy has led to a significant increase in progression free survival (PFS), although including both substances in first line treatment does not further increase PFS.[1]

However, the prognosis for patients with metastatic colorectal cancer patients is still limited. Moreover, many patients suffer from severe toxic side effects of chemotherapy. It would be useful to identify patients who are most likely to benefit from a specific chemotherapeutic regimen, as well as those who may experience severe adverse reactions. Clinical parameters alone have proven to be inadequate in predicting chemosensitivity. Pharmacogenetics aims at developing germline genetic markers to be used for predicting pharmacological response in the individual patient.[2] This review presents recent developments in pharmacogenetic studies in chemotherapy of colorectal cancer.

5-Fluorouracil (5-FU)

The fluoropyrimidine derivative 5-fluorouracil (5-FU) is thought to have two major mechanisms of action to explain its cytotoxic effect. Most importantly, the active metabolite of 5-FU (5-FdUMP) prevents DNA synthesis by forming a complex with thymidylate synthase (TS) that is stabilized by 5,10-methylenetetrahydrofolate (5,10-MTHF), thereby inhibiting the conversion of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP), the latter of which is an essential precursor for DNA-synthesis. (Figure 1) Furthermore, incorporation of 5-FU nucleotides into DNA and RNA leads to altered RNA processing and DNA damage.

Thymidylate synthase (TS)

The gene encoding for TS contains a unique tandemly repeated 28bp sequence in the enhancer region (TSER) in the 5'-untranslated region (5'-UTR), that was shown to be polymorphic with regard to the number of repeats (variable number of tandem repeats, VNTR). Although alleles containing up to 9 repeats have been described, two (2R) and three (3R) repeat copies are the most prevalent alleles in all ethnic populations.[3;4] The 3R-allele leads to increased tumoral TS expression, due to either enhanced mRNA translation efficiency[5] or increased *TS* mRNA

levels.[6] In addition to this VNTR polymorphism, a single nucleotide polymorphism (SNP) G>C at bp12 of the second repeat in 3R individuals has been described, leading to a three-allelic locus (2R, 3RC, 3RG). The 3RC allele leads to a reduced transcriptional activity comparable to that of the 2R allele, by disrupting an area critical to *TS* promoter activation.[4]

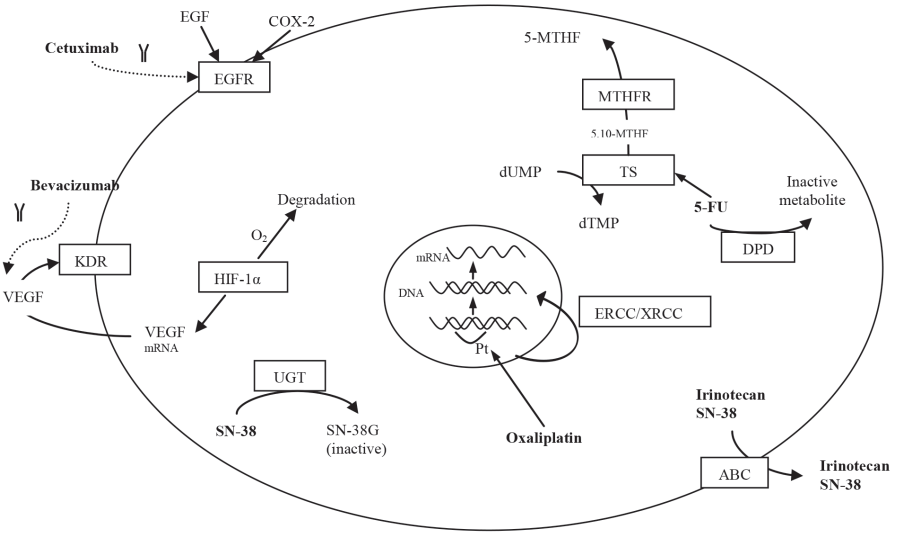

Figure 1. Schematic simplified overview of enzymes involved in the cellular response and metabolism of chemotherapeutic compounds in colorectal cancer

5-FU: 5-fluorouracil; ABC: ATP-binding cassette; DPD: Dihydropyrimidine dehydrogenase; dTMP: deoxythymidine monophosphate; dUMP: deoxyuridine monophosphate; EGFR: endothelial growth factor receptor; ERCC1: Excision repair cross complementation group 1; HIF-1α: hypoxia inducible factor 1α ; KDR: Kinase domain receptor; MTHF Methylene hydrofolate; MTHFR: Methylene hydrofolate reductase; Pt: Platinum; SN-38: active metabolite of irinotecan; SN-38G: SN-38 glucoronide; TS: Thymidylate synthase; UGT: UDP-glucuronosyl transferase; VEGF: vascular endothelial growth factor; XRCC1: X-ray repair cross complementation group 1.

In several studies, carriers of the 3R allele showed a poorer response to 5-FU chemotherapy[6-9], as well as decreased rates of grade 3-4 overall toxicity[3;6] and diarrhea[10], as would be expected from the higher TS levels associated with this allele.(Table 1) Conversely, two independent studies found a significantly better response rate and survival for 3R3R homozygotes, compared with individuals carrying at least one 2R allele.[11;12] The C>G SNP has frequently been used to explain the discrepancies in studies addressing only the VNTR polymorphism. The 3RG genotype was most often associated with either shorter response duration[13;14], shorter OS[14;15] or reduced overall response[14] to 5-FU, when compared to 2R or 3RC genotypes. However, although a lower rate of toxicity for 3RG3RG individuals was found in some studies[3;11], another study found a trend towards more toxicity during the first cycle of capecitabine treatment.[13]

Another explanation for these discrepant findings may be the ethnic diversity in relative allele frequencies, with only 3-4% 2R homozygotes in most Asian populations, compared to 17-24% in Caucasians.[3;6]

In addition to the TSER polymorphism another polymorphic locus is found in the *TS* 3'-UTR, consisting of a 6bp deletion at position 1494.[16] The del6 allele has been associated with better response rate[17;18] and reduced risk of death[19], although in another study ins6 homozygotes showed significantly better response to capecitabine or raltitrexed.[9] Regarding adverse reactions, although several studies showed the 3'-UTR polymorphism had no influence on toxicity[3;13;20], one study found a higher incidence of toxicity in del6 homozygotes.[18]

Methylenetetrahydrofolate reductase (MTHFR)

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion MTHF to 5-methyltetrahydrofolate. The former is essential in stabilizing the complex formed by TS and 5-FdUMP. (Figure 1)

The *MTHFR* gene is subject to several polymorphisms. Most common are two SNPs, 677C>T (Ala222Val, *MTHFR**4) and 1298A>C (Glu428Ala, *MTHFR**6), that are in linkage disequilibrium.[3;13;21] These polymorphisms lead to decreased MTHFR enzyme activity, and may thereby induce more effective stabilization of the FdUMP-TS ternary complex. In vivo studies showed a significantly better response rate for genotypes with at least one *MTHFR* 677T allele.[12;22](Table 1) Conversely, a study in 142 patients with primary rectal adenocarcinoma showed increased tumor regression for *MTHFR* 677CC homozygotes after preoperative 5-FU based chemoradiation therapy.[21] However, most studies found that both the *MTHFR* 677C>T and *MTHFR* 1298A>T polymorphisms were not predictive of objective response or survival in patients treated with 5-FU chemotherapy.[12;20;23;24] Enhanced stability of the ternary complex could also be expected to increase 5-FU toxicity. However, a recent study unexpectedly found lower rates of toxicity for the *MTHFR* 677TT and 1298AA genotypes.[20] Most studies found both *MTHFR* variants were not associated with altered toxicity rates in diverse patient groups treated with 5-FU derivatives.[13;22;23]

Dihydropyrimidine dehydrogenase (DPD)

5-FU depends on the activity of dihydropyrimidine dehydrogenase (DPD) for 80% of its catabolism.[25] Deficiency of DPD-activity leads to prolongation of the plasma half-time with resulting accumulation of 5-FU and has been associated with severe, mainly hematological toxicity and even death after administration of 5-FU.[26] DPD deficiency is present in approximately 3% of all cancer patients[27] but accounts for approximately 50% of patients with unexpected severe 5-FU toxicity.[28]

To date, over 30 polymorphisms in the *DPYD* gene (encoding for DPD) have been identified, many of which were found to be common variants with no apparent effect on DPD-activity.[29;30] However, a G>A mutation of the invariant splice site in exon 14 (IVS14+1G>A, *DPYD**2A) leads to skipping of exon 14 and formation of a truncated protein with no apparent residual

activity. Homozygosity for *DPYD**2A can lead to complete DPD-deficiency.[26] Although the incidence of this allele is rare, with a population frequency of 0,9-1,8% heterozygotes[9;26;31], it is estimated to be responsible for about 25% of all cases of unexpected severe 5-FU-toxicity.[10;28;31] However, it has also been described in individuals with normal DPD-activity.[28] There are profound ethnic differences in the incidence of DPD gene mutations. Although *DPYD**2A is the most common polymorphism in Caucasians, it is rare in Asians.[29]

Irinotecan

Irinotecan (CPT-11), a potent inhibitor of topoisomerase I, is widely used in the therapy for various solid tumors, including advanced colorectal carcinoma. Irinotecan undergoes several metabolic steps, both anabolic and catabolic.(Figure 1) It undergoes biotransformation by CYP3A-mediated oxidation to form APC, a substance which shows little cytotoxic activity. By another route, it is converted by liver carboxylesterases to its active metabolite (SN-38). This compound is then further conjugated by several UDP-glucuronyltransferases (UGTs) to yield the inactive SN-38G, which is mainly excreted with bile and urine. To enable excretion, SN-38 and irinotecan are actively transported out of the cell by an ATP-dependent efflux pump (ABC-binding cassette B1, ABCB1). After biliary excretion, SN-38G can be converted to active SN-38 by bacterial β-glucuronidase, which can lead to gastro-intestinal toxicity. Finally, SN-38 is subject to enterohepatic recirculation, leading to an unexpected peak in plasma after gall bladder emptying.

UDP-glucuronosyltransferase (UDP)

Reduced glucuronidation of SN-38 has been shown to significantly increase gastro-intestinal toxicity of irinotecan.[32] The principle UGT involved in the conjugation of SN-38 is UGT-1A1.[33] Up to 25 polymorphisms have been described for *UGT1A1*[34], of which a polymorphism in the promoter region, consisting of seven instead of six TA-repeats (-53$(TA)_{6>7}$, *UGT1A1**28) is the most common. The higher number of TA-repeats is associated with reduced transcriptional activity of *UGT1A1*, leading to various degrees of impaired glucuronidation.[35-39] This allele is also associated with Gilbert's syndrome.[40] The presence of the *UGT1A1**28 allele in either heterozygote or homozygote form, was shown to be a significant predictor for severe toxicity after the admission of irinotecan[41], with up to seven times increased risk of severe neutropenia[37;39;42;43] or diarrhea[36;42;43], and a severely increased risk of febrile neutropenia.[44] This increase in toxicity, however, may only be present in the first cycle of chemotherapy.[39] Although most studies have shown no significant association between *UGT1A1*-genotype and objective response[42-44], a study in 250 white patients with advanced colorectal cancer showed a higher incidence of partial or complete response for *UGT1A1**28 patients.[39]

Although, with a population frequency of 43% heterozygotes, the *UGT1A1**28 polymorphism is the most frequent mutation in Caucasians[36;38], it is significantly less frequent in

Asians.[45;46] Contrary, in the Asian population, the most common polymorphism is a SNP in the 3'-UTR (211G>A, *UGT1A1**6)[45], which is also associated with significantly reduced enzyme activity.[47] One study showed a higher incidence of grade 4 neutropenia after administration of irinotecan for *UGT1A1**6 homozygotes, combined with a significantly lower response rate and shorter PFS.[45] Conversely, most studies showed no increased risk of toxicity for the *UGT1A1**6 allele.[42]

Two other UGT isoenzymes are thought to be involved in the glucuronidation of SN-38: the hepatic UGT1A9 and the extra-hepatic UGT1A7.[48] The -118 in/del polymorphism (-118(T)$_{9>10}$, *UGT1A9**22), showing higher *UGT1A9* mRNA expression for the -118(T)9 allele, might explain part of the phenotypic variability in SN-38 glucuronidation that is not explained by *UGT1A1**28.[46]

Oxaliplatin

Platinum-containing drugs exert their cytotoxic effect by forming bulky interstrand and intrastrand DNA-adducts, resulting in DNA replication inhibition and apoptosis. The major pathway for removal of these adducts is nucleotide excision repair (NER).(Figure 1) During NER, damaged DNA is recognised and DNA helixes are unwound by the action of several factors, including xeroderma pigmentosum complementation group D protein (XPD, also known as ERCC2), XPC and XPA. The DNA strands are separated and a DNA residue containing the adducts is removed. Cleaving of the damaged strand is performed by the nucleases XPG and excision repair cross-complementing group 1 protein (ERCC1) on the 3' and 5' side, respectively. Suboptimal repair mechanisms may lead to increased sensitivity to platinum containing chemotherapy.[49] Higher tumoral *ERCC1* mRNA levels have been associated with significantly worse outcome in gastric cancer[50] and advanced colorectal cancer[51] treated with cisplatin or oxaliplatin based chemotherapy. Polymorphisms have been described for many of the constituents of NER. However, to date, only few have proven clinically significant.

Xeroderma pigmentosum complementation group D (XPD)

The synonymous *XPD* Arg156Arg (C>A) polymorphism was associated with a higher response rate and longer time to progression for C/A or A/A genotypes in gastric cancer patients treated with oxaliplatin based chemotherapy.[17] A trend towards higher response and longer median survival rate for these genotypes was also seen in metastasized colorectal cancer patients.[52](Table 1) Another polymorphism, *XPD* Lys751Gln (A>C), was shown to reduce DNA repair capacity for the 751Gln/Gln genotype in normal cells of lung cancer patients.[53] Conversely, a reduced capacity for DNA repair for 751Lys/Lys genotype was found in another report, possibly because of methodological differences.[54] The *XPD* Lys571Gln polymorphism did not show significant survival difference according to genotype in gastro-oesophageal cancer[14;55] and colorectal cancer[56;57] in response to various platinum based chemotherapy regimens.

Excision repair cross complementing group 1 (ERCC1)

A synonymous C>T (Arg118Arg) polymorphism in the *ERCC1* gene has been described. Although the mechanism by which this substitution affects ERCC1 activity is unknown, it has been suggested that replacement of the common codon AAC by the infrequently used codon AAT affects translation efficiency, with a 50% decrease for the variant allele.[58] Advanced colorectal cancer patients carrying the -118TT genotype experienced higher response rates to oxaliplatin treatment[59] and longer progression free survival[60] in two studies.(Table 1) However, in another two studies survival was most favorable for patients who carried the *ERCC* -118CC genotype.[23;61] Two studies in gastric cancer patients found no predictive effect of this polymorphism.[14;17]

X-ray cross complementing group 1 (XRCC1)

In addition to NER, the basepair excision repair pathway (BER) is also involved in chemosensitivity to platinum agents. An important player in BER is X-ray repair cross complementing group 1 (XRCC1). A common polymorphism, *XRCC1* Arg399Gln (G>A), has been suggested to produce significant conformational changes at a domain important for XRCC1 interaction with other components of BER.[62] The germline wildtype allele has been associated with significant survival benefit in gastric cancer patients[55] and lung cancer patients[63] in response to platinum compounds.(Table 1) Expression of the wildtype allele in colorectal tumoral tissue was also associated with better survival and response to oxaliplatin.[24] However, in recent studies in advanced colorectal cancer and gastric cancer patients, *XRCC1* genotype did not predict outcome after oxaliplatin treatment.[23;56;61]

Cetuximab

Cetuximab, a chimeric immunoglobin G1 monoclonal antibody, exerts is action by binding to the extracellular domain of the epidermal growth factor receptor (EGFR) with a higher affinity than epidermal growth factor (EGF), thereby blocking ligand-induced phosphorylation of EGFR.(Figure 1) So far, only clinical parameters have been used to predict cetuximab efficacy, of which the grade of skin toxicity is the most important.[64] However, pretreatment markers for selecting patients who may benefit from therapy are currently lacking. EGFR-staining intensity in tumor tissue is not associated with response, survival or toxicity.[65-67] Pharmacogenetics may prove a possible way of optimizing monoclonal antibody therapy.[68]

Epithelial growth factor receptor (EGFR)

A polymorphic $(CA)_n$-repeat variant in *EGFR* intron-1 has been described with 16 up to 26 repeats and *EGFR* gene transcription declines with increasing number of (CA)-repeats.[69] In a study in 110 heavily pretreated patients with advanced colorectal carcinoma, the homozygous *EGFR* intron-1 short allele was associated not only with favorable survival, but also with a higher grade of skin toxicity.[64](Table 1) In an earlier study, however, no relation between this

polymorphism and colorectal cancer survival was detected.[67] Another polymorphic locus in the *EGFR* gene has been described, consisting of a SNP G>A leading to substitution of arginine by lysine at codon 497 (also denominated 521). This polymorphism was shown to be predictive of cetuximab efficacy, with a better response rate as well as longer PFS and OS for advanced colorectal cancer patients carrying at least one A allele.[70;71] Earlier studies, however, showed no influence of this and another (*EGFR* -216G>T) polymorphism on cetuximab efficacy.[64;67]

Induction of downstream pathways of EGFR leads to synthesis of various ligands, such as Cyclin-D1 (CCND1). Therefore, genetic variation in the cyclin-D1 gene might affect cetuximab efficacy, and germline polymorphisms have been described for *CCND1*. In metastatic colorectal cancer patients receiving cetuximab single-agent therapy, harboring the SNP (*CCND1* 870 A>G) is associated with longer OS for the G allele.[67](Table 1) Conversely, in another study in advanced colorectal patients treated cetuximab and irinotecan combination therapy, this polymorphism was not associated with PFS or OS.[64] In addition, Cox-2 acts as an upstream regulator of EGFR. A frequent *Cox2* -756G>C polymorphism has been described, leading to decreased COX-2 expression.[72] Until recently, this polymorphisms had not been related to response or outcome in patients treated with cetuximab[67], but in a recent study *Cox-2* -756CC individuals showed longer PFS.[71]

A difference in the expression of the natural ligand for the EGFR might also influence cetuximab efficacy. A SNP is found in the *EGF* gene 61G>A, which leads to upregulated EGF levels for the transcriptionally more active G allele. Although higher circulating EGF levels have been associated with higher tumor aggressiveness, the *EFG* 61GG genotype was associated with a more favorable overall survival in patients treated with cetuximab and irinotecan.[64;71] However, in another study AA homozygosity tended to associate with longer overall survival.[67]

Antibody-dependent cell-mediated cytotoxicity

Cetuximab may also exert an indirect anti-tumor activity by attracting cytotoxic host effector cells, like monocytes and natural killer cells. The effect of this antibody-dependent cell-mediated cytotoxicity (ADCC) may depend on the degree of activation of effector cells after engagement of immunoglobin G fragment C receptors (FcγR) IIa and IIIa. Polymorphic alleles have been described for *FcγR-IIIa* (559T>G, Val158Phe) and *FcγR-IIa* (535G>A, His131Arg), that were shown to negatively affect receptors' affinities for the fragment C of antibodies, and probably ADCC efficiency.[73] *FcγR-IIIa* 158VV genotype was associated with a higher affinity of natural killer cells for the chimeric IgG1 monoclonal antibody rituximab in vitro[74], and with a higher response rate and longer progression free survival in breast cancer patients treated with the humanized anti-Her2/neu immunoglobin G monoclonal antibody trastuzumab.[75] (Table 1) A study in 39 mCRC patients treated with cetuximab monotherapy unexpectedly showed a significantly shorter PFS for *FcγR-IIa* 131A and *FcγR-IIIa* 158V homozygotes.[76] However, another study in advanced colorectal patients treated with irinotecan an cetuximab, showed neither polymorphism was associated with PFS or OS.[64]

Bevacizumab

Bevacizumab is a humanized recombinant monoclonal IgG type antibody, directed against all VEGF-A isoforms.(Figure 1) VEGF is an important regulator of angiogenesis and its inhibition by bevacizumab not only reduces tumor volume, but also large vessel density in a colorectal tumor model in mice.[77] Hypoxia is a potent stimulus for VEGF expression and one of the regulating elements in this mechanism is hypoxia-inducible factor 1α (HIF-1α). This factor binds to a 28-bp enhancer in the 5' upstream region of the *VEGF*-gene, thereby stimulating transcription. Under normoxic cellular conditions, HIF-1α rapidly degrades. However, it may be strikingly induced under hypoxic conditions, which are often found in tumor mass. In addition to HIF-1α, other regulating elements for VEGF expression are located in the 3'-UTR.

Vascular endothelial growth factor

Several polymorphisms have been described for the *VEGF* promoter region, 5'-UTR and 3'-UTR[78;79], but only few have shown functional implications.[80] Two SNP's (*VEGF* +936C>T and *VEGF* -1154G>A) lead to decreased VEGF expression for the variant allele.[78;81-84] A third SNP (*VEGF*-2578C>A), that is in complete linkage disequilibrium with an 18bp insertion at bp -2549, has also been associated with higher VEGF expression and serum levels for the wildtype -2578C/-2549del allele.[78;79] However, lower VEGF expression for the *VEGF*-2578CC genotype was found in one study.[78;81] Another common polymorphism (*VEGF* -634G>C, also denominated +405G>C) was most commonly reported to induce lower VEGF expression and serum protein levels for wildtype homozygotes[81;83;85], although one study conversely found decreased VEGF expression for the variant allele.[86] In addition, recent studies found no association between these *VEGF* polymorphisms and tumoral VEGF protein expression or circulating VEGF levels.[87;88] Methodological differences may have contributed herein, since one report found increased *VEGF* mRNA expression in colorectal carcinoma tissue, but not in adjacent healthy tissue.[89]

So far, only one study on the pharmacogenetic interaction between bevacizumab and *VEGF* polymorphisms has been published. A recent study in 363 breast cancer patients found improved median survival time for patients with the *VEGF* -2578AA and -1154AA genotypes when treated with paclitaxel combined with bevacizumab.[90] Instead, most studies have focused on prognostic, rather than predictive effects. *VEGF* -634CC genotype was associated with higher tumor stage and grade in one study in breast cancer patients[91], but with increased OS in another.[92] In colorectal cancer patients, this polymorphism was not associated with tumor differentiation[93] or time to tumor recurrence.[94] In early stage gastric carcinoma the *VEGF*-460CC genotype was associated with a better DFS and OS[87], but the same genotype was associated with reduced OS in Chinese breast cancer patients.[92] Stage III colorectal cancer patients with the *VEGF* +936CC homozygote genotype had significantly shorter time to tumor recurrence compared to patients with at least one T allele.[94] *VEGF* -2578CC homozygotic patients with renal cell carcinoma showed significantly lower cancer specific survival, compared

to patients with at least one variant allele.[95] Another polymorphism *VEGF* -1498T>C showed poorer differentiation of colorectal carcinomas for the CC_genotype.[93] These conflicting and sometimes unexpected results concur with our current lack of full understanding of these polymorphisms on VEGF expression and function.

Hypoxia-inducible factor 1α

Three polymorphisms have been described in exon 12 of the *HIF-1α* gene, *HIF-1α* Pro582Ser (1722C>T), *HIF-1α* Ala588Thr (1790G>A), *HIF-1α* Pro564Ala, and one in exon 13, which is a GT-repeat polymorphism.[96;97] Genotypes coding for variant proteins showed higher transcription capacity in vitro, both under normoxic and hypoxic conditions, compared to wildtype.[97;98] Expression of a Pro582Ser variant allele was associated with a significantly increased risk of ulcerative disease in colorectal cancer, although vascularization was not increased.[99] Whereas both Pro582Ser and Ala588Thr were not associated with tumor grade or stage in transitional cell carcinoma, patients with at least one variant allele showed significantly worse disease-free and overall survival.[100]

Discussion

So far, pharmacogenetic studies hold the promise of becoming a useful way of predicting results for chemotherapeutic treatment in colorectal carcinoma. Results from earlier trials have even lead to a label change for irinotecan, now advising dose reduction for *UGT1A1*28* homozygotic individuals. There are, however, some difficulties in interpreting study results.

Pharmacogenetic studies aim at understanding the influence of germline polymorphisms. This does not account for the potential bias of somatic mutations or loss of heterozygosity in the tumor, which is a frequent phenomenon with regard to the *TYMS* polymorphisms(26-54%).[3;19] Patients who have a 2R3R genotype, with tumoral 3R-loss might obtain significant benefit from 5-FU based chemotherapy, with a lower risk of toxicity. In addition, the frequent presence of linkage disequilibrium between variant alleles makes it difficult to ascertain which allele is essential in predicting chemosensitivity. Haplotype analysis may eventually overcome this problem.

Furthermore, ethnic differences in relative allele frequencies may want for different strategies for the respective populations. Substantial interethnic differences have been found for the allele frequencies of *ERCC1* 118, *XRCC1* 399 and *XPD* 751 polymorphisms, with African Americans carrying the wildtype allele more often than Americans of European descent.[101] Asian populations show only very limited expression of the beneficial *TYMS* 2R2R genotype, and a different spectrum of *UGT1A1* polymorphisms, compared to Caucasians.[102;103]

Another problem regarding pharmacogenetic results lies in the fact that some polymorphisms are not only predictive of chemotherapeutic efficacy, but also of general prognosis in cancer patients[104] and may even have contributed to the development of colorectal

carcinoma[105]. This is especially true for components of DNA repair mechanisms. By impairing DNA repair polymorphic changes may predispose to carcinogenesis, whereas they may also improve response to chemotherapy when cancer has developed.

In developing predictive pretreatment models for colorectal cancer therapy genetic and non-genetic factors with proven effect on outcome, such as performance status and tumor stage will need to be combined. This has proven effective in other fields, such as predicting MTX treatment efficacy rheumatoid arthritis.[106]

Conclusion

In conclusion, pharmacogenetic studies in colorectal cancer therapy show promising results with regard to prediction of tumor response, survival and toxicity. Although further research is warranted, predictive models including genotypic testing will influence the choice for a chemotherapy regimen in the future.

First Author	Ethnicity	Cancer type	Study design	Gene studied	Polymorphism	Effect on toxcity	Effect on outcome	Reference
Pullarkat S.T. 2001	Mixed	mCRC	Retrospective	TS	TSER	3R/3R decreased grade 3 toxicity (27% vs 63%)	3R/3R decreased RR (9% vs 50%)	6
Salgado J.2007	NS (Spanish)	mCRC	Prospective	TS	TSER	NA	3R/3R decreased RR (7/33 vs 13/15)	9
					Ins/del 6bp	NA	Del6/del6 decreased RR (0/11 vs 24/34)	
Park D.J. 2002	20/24 Caucasian	mCRC	Prospective	TS	TSER	3R/3R decreased grade 3 toxicity (37.5% vs 100%, p 0.17)	3R/3R decreased RR (25% vs 75%)	8
Lecomte T. 2004	NS (French)	CRC	Retrospective	TS	TSER, C>G	3R (C or G) decreaed grade 3-4 toxicity (43% vs 4%)	No effect of polymorphism	3
					Ins/del 6bp	No effect of polymorphism	No effect of polymorphism	
Marsh S. 2001	Caucasian	CRC	?	TS	TSER	NA	3R/3R decreased RR + MST (HR 2.33, 12 vs 16 months)	7
Schwab M. 2008	NS (German)	Mixed	Prospective	TS	TSER	3R/3R decreased risk of diarrhoea (OR 0.36)	NA	10
Jakobsen A. 2005	NS (Danish)	mCRC	Retrospective	TS	TSER	NA	3R/3R increased RR (52% vs 24%)	12
Hitre E. 2005	NS (Hungary)	CRC	Prospective	TS	TSER	3R/3R decreased toxicity (22% vs 30%)	3R/3R increased OS (HR 3.79)	11
Largillier R. 2006	NS (French)	Breast cancer	Prospective	TS	TSER, C>G	3G/3G increased grade 3-4 toxicity 1st cycle (p 0.064)	3G/3G longer response duration	13
					Ins/del 6bp	No effect of polymorphism	No effect of polymorphism	
Ruzzo A. 2006	NS (Italian, Japanese)	AGC	Prospective	TS	TSER, C>G	NA	3G allele decreased OS and PFS (RR 2.21 and 2.16)	14
Marcuello E. 2004	NS (Spanish)	mCRC	Prospective	TS	TSER, C>G	NA	3G allele decreased OS and RR (HR 2.4 and OR 2.9)	15

First Author	Ethnicity	Cancer type	Study design	Gene studied	Polymorphism	Effect on toxcity	Effect on outcome	Reference
Lu J.W. 2006	Chinese	AGC	Prospective	TS	Ins/del 6bp	Del6/del6 increased grade 3-4 toxicity	Del6 allele increased RR (37% vs 0%)	18
Keam B. 2008	Korean	AGC	Prospective	TS	Ins/del 6bp	No effect of polymorphism	Del6/del6 increased RR (55% vs 30,3%)	17
Dotor E. 2006	NS (Spanish)	CRC	Prospective	TS	Ins/del 6bp	NA	Del6 increased OS (HR 0.47)	19
Sharma R. 2008	NS (Australia)	mCRC	Prospective	TS	Ins/del 6bp	No effect of polymorphism	No effect of polymorphism	20
Jakobsen A. 2005	NS (Danish)	mCRC	Retrospective	MTHFR	677C>T	NA	T allele increaed RR (67% vs 34%)	12
					1298A>C	NA	No effect of polymorphism	
Largillier R. 2006	NS (French)	Breast cancer	Prospective	MTHFR	677C>T	No effect of polymorphism	No effect of polymorphism	13
					1298A>C	No effect of polymorphism	No effect of polymorphism	
Sharma R. 2008	NS (Australia)	mCRC	Prospective	MTHFR	677C>T	T/T decreased grade 2-3 toxicity (OR 0.1)	No effect of polymorphism	20
					1298A>C	A/A decreased grade 2-3 toxicity (32% vs 72%)	No effect of polymorphism	
Terrazino S. 2006	NS (Italian)	Rectal cancer	Prospective	MTHFR	677C>T	NA	C/C increased RR (57% vs 34%, OR 0.32)	21
Cohen V. 2003	38/43 Caucasian	mCRC	Prospective	MTHFR	677C>T	No effect of polymorphism	T allele increased RR (OR 2.86)	22
Ruzzo A. 2007	NS (Italian)	mCRC	Prospective	MTHFR	677C>T	No effect of polymorphism	No effect of polymorphism	23
					1298A>C	No effect of polymorphism	No effect of polymorphism	
Suh K.W. 2006	Korean	mCRC	Retrospective	MTHFR	677C>T	NA	No effect of polymorphism	24
Innocenti F. 2004	50/66 Caucasian	mCRC	Prospective	UGT1A1	53$(TA)_{6>7}$	TA_7 increased grade 4 neutropenia (3/6 vs 0/29)	NA	37
Toffoli G. 2006	NS (Italian)	mCRC	Prospective	UGT1A1	53$(TA)_{6>7}$	TA_7 increased grade 3-4 neutropenia (OR 8.63)	TA7 decreased risk of progression (OR 0.19)	39

First Author	Ethnicity	Cancer type	Study design	Gene studied	Polymorphism	Effect on toxcity	Effect on outcome	Reference
De Jong F.A. 2006	NS (Dutch)	CRC and GC	Prospective	UGT1A1	$53(TA)_{6>7}$	TA_7 increased grade 2-3 diarrhoea (69.2% vs 34.6%)	NA	36
Kweekel D.M. 2008	NS (Dutch)	mCRC	Prospective	UGT1A1	$53(TA)_{6>7}$	TA_7 increased febrile neutropenia (OR 14.2)	No effect of polymorphism	41
Marcuello E. 2004	NS (Spanish)	mCRC	Prospective	UGT1A1	$53(TA)_{6>7}$	TA_7 increased diarrhoea (7/10 vs 7/40)	No effect of polymorphism	43
Ando Y. 2000	Japanese	Mixed	Retrospective	UGT1A1	$53(TA)_{6>7}$	TA_7 increased grade 4 toxicity (4/7 vs 14/93)	NA	42
					211G>A	No effect of polymorphism	NA	
Han J.Y. 2006	Korean	NSCLC	Prospective	UGT1A1	211G>A	211A increased grade 4 neutropenia (4/6 vs 18/75)	211A lower RR (0/5 vs 36/72)	45
Minami H. 2006	Japanese	NS	Prospective	UGT1A1	$53(TA)_{6>7}$ /211G>A	TA_7/211A increased neutropenia (4/5 vs 3/21)	NA	107
Stoehlmacher J. 2004	75/106 Caucasian	mCRC	Retrospective	XPD	Lys751Gln	NA	Gln/Gln increased risk of dying (OR 2.44)	61
Keam B. 2008	Korean	AGC	Prospective	XPD	Arg156Arg	No effect of polymorphism	A/A increased RR (52% vs 26,1%)	17
Ruzzo A. 2007	NS (Italian)	mCRC	Prospective	XPD	Lys751Gln	No effect of polymorphism	Gln/Gln decreased PFS (HR 2.21)	23
					Asp312Asn	No effect of polymorphism	No effect of polymorphism	
Park D.J. 2001	55/73 Caucasian	mCRC	Prospective	XPD	Lys751Gln	NA	Gln/Gln decreased MST (17,4 vs 3,3 months)	52
					Asp312Asn	NA	No effect of polymorphism	
					Arg156Arg	NA	A/A decreased RR (24 vs. 13%, p 0.69)	
Gurubhagavatula S. 2004	NS (US)	NSCLC	Prospective	XPD	Asp312Asn	NA	Asn/Asn decreased OS (HR 2.18)	63
Liu B. 2007	Chinese	Gastric cancer	Prospective	XPD	Lys751Gln	NA	No effect of polymorphism	55

First Author	Ethnicity	Cancer type	Study design	Gene studied	Polymorphism	Effect on toxcity	Effect on outcome	Reference
Ruzzo A. 2006	NS (Italian, Japanese)	AGC	Prospective	XPD	Lys751Gln	NA	No effect of polymorphism	14
Monzo M. 2007	NS (Spanish)	mCRC	Prospective	XPG	Lys751Gln	No effect of polymorphism	No effect of polymorphism	57
					Hys46Hys	No effect of polymorphism	C/C increased RR (CR+PR 74% vs 34%)	
Braun M.S. 2008	NS (UK)	mCRC	Prospective	XPD	Lys751Gln	NA	No effect of polymorphism	56
Ruzzo A. 2006	NS (Italian, Japanese)	AGC	Prospective	ERCC1	Asn118Asn	NA	No effect of polymorphism	14
Keam B. 2008	Korean	AGC	Prospective	ERCC1	Asn118Asn	No effect of polymorphism	No effect of polymorphism	17
Ruzzo A. 2007	NS (Italian)	mCRC	Prospective	ERCC1	Asn118Asn	No effect of polymorphism	T/T decreased PFS (HR 2.34)	23
Stoehlmacher J. 2004	75/106 Caucasian	mCRC	Retrospective	ERCC1	Asn118Asn	NA	T/T increased risk of dying (OR 1.86)	61
Viguier J. 2005	NS (French)	mCRC	Prospective	ERCC1	Asn118Asn	NA	T/T increased response (OR 3.03)	59
Martinez-Balibrea E. 2008	Caucasian	mCRC	Prospective	ERCC1	Asn118Asn	NA	C allele decreased PFS (HR 1.96, p 0.0501)	60
Ruzzo A. 2007	NS (Italian)	mCRC	Prospective	XRCC1	Arg399Gln	No effect of polymorphism	No effect of polymorphism	23
Gurubhagavatula S. 2004	NS (US)	NSCLC	Prospective	XRCC1	Arg399Gln	NA	Gln/Gln decreased OS (HR 2.88)	63
Liu B. 2007	Chinese	Gastric cancer	Prospective	XRCC1	Arg399Gln	NA	Gln/Gln decreased MST (212 vs 347 days)	55
Braun M.S. 2008	NS (UK)	mCRC	Prospective	XRCC1	Arg399Gln	NA	No effect of polymorphism	56
Stoehlmacher J. 2004	75/106 Caucasian	mCRC	Retrospective	XRCC1	Arg399Gln	NA	No effect of polymorphism	61
Suh K.W. 2006	Korean	mCRC	Retrospective	XRCC1	Arg399Gln	NA	Gln/Gln decreased MST (12.8 vs 30 months)	24
Graziano F. 2008	NS	mCRC	Prospective	EGFR	$(CA)_n$-repeat	$(CA)_{\leq 17}$ increased grade 2-3 skintoxicity	$(CA)_{\leq 17}$ increased OS (HR 0.54)	64
					497G>A	No effect of polymorphism	No effect of polymorphism	
					216G>T	No effect of polymorphism	No effect of polymorphism	

First Author	Ethnicity	Cancer type	Study design	Gene studied	Polymorphism	Effect on toxcity	Effect on outcome	Reference
Zhang W. 2006	31/39 Caucasian	mCRC	Prospective	EGFR	$(CA)_n$-repeat	No effect of polymorphism	No effect of polymorphism	67
Nagashima F. 2007	NS (US)	mCRC	Prospective	EGFR	497G>A	NA	GA increased PFS (1.8 vs 1.2 months)	71
Goncalves A. 2008	NS (French)	mCRC	Retrospective	EGFR	497G>A	NA	A allele increased PFS and OS (5.7/20.1 vs 3.1/13.8 months)	70
Graziano F. 2008	NS	mCRC	Prospective	CCND1	870A>G	No effect of polymorphism	No effect of polymorphism	64
Zhang W. 2006	31/39 Caucasian	mCRC	Prospective	CCND1	870 A>G	No effect of polymorphism	A/A decreased OS (2.3 vs 7.8 months)	67
Zhang W. 2006	31/39 Caucasian	mCRC	Prospective	EGF	61G>A	No effect of polymorphism	AA increased PFS (15.0 vs 2.3 months, *p* 0.07)	67
Nagashima F. 2007	NS (US)	mCRC	Prospective	EGF	61G>A	NA	G/G increased PFS (1.4 vs 1.2 months)	71
Graziano F. 2008	NS	mCRC	Prospective	EGF	61A>G	No effect of polymorphism	EGF 61GG increased OS (HR 0.55)	64
Zhang W. 2006	31/39 Caucasian	mCRC	Prospective	COX-2	-756G>C	No effect of polymorphism	No effect of polymorphism	67
Nagashima F. 2007	NS (US)	mCRC	Prospective	COX-2	-756G>C	NA	C/C increased PFS (6.9 vs 1.3 months)	71
Lurje G. 2008	70/125 Caucasian	CRC	Prospective	VEGF	936C>T	NA	T/T increased time to recurrence (11 vs 2.6 years)	94
				IL-8	-251T>A	NA	A/A decreased time to re-currence (2.4 vs 5.7 years)	
Graziano F. 2008	NS	mCRC	Prospective	FCGR IIa	His131Arg	No effect of polymorphism	No effect of polymorphism	64
				FCGR IIIa	Val158Phe	No effect of polymorphism	No effect of polymorphism	
Musolino A. 2008	NS (Italian)	Breast cancer	Prospective	FCGR IIa	His131Arg	NA	His/His increased RR (70% vs 40%)	75
				FCGR IIIa	Val158Phe	NA	Val/Val increased RR (82% vs 35%)	
Zhang W. 2007	31/39 Caucasian	mCRC	Prospective	FCGR IIa	His131Arg	NA	Arg/Arg decreased PFS (1.1 vs 2.4 months)	67

First Author	Ethnicity	Cancer type	Study design	Gene studied	Polymorphism	Effect on toxcity	Effect on outcome	Reference
				FCGR IIIa	Val158Phe	NA	Phe/Phe increased PFS (2.3 vs 1.1 months)	
Kim J.G. 2007	Korean	GC	Prospective	VEGF	-460T>C	NA	C allele increased DFS (HR 4.87)	87
Jin Q. 2005	NS (Europe)	Breast cancer	Prospective	VEGF	-634G>C	NA	-634CC higher tumor stage/grade	91
Lu H. 2005	Chinese	Breast cancer	Prospective	VEGF	-460T>C	NA	C/C decreased OS (HR 1.5)	92
Yamamori M. 2008	Japanese	CRC	In vitro	VEGF	-1498T>C	NA	C/C poorer tumor differentiation	89
					-634G>C	NA	No effect of polymorphism	
Kawai Y. 2007	Japanese	Renal cell carcinoma	Prospective	VEGF	-2578C>A	NA	A allele increased CSS (HR 0.71)	95
Schneider B. 2008	NS (Canada)	Breast cancer	Retrospective	VEGF	-2578C>A	No effect of polymorphism	A/A increased MST (HR 1.70)	90
					-1154 G>A	No effect of polymorphism	A/A increased MST (HR 2.69)	
					-1498T>C	T/T decreased grade 3-4 hypertension (8% vs 23%)	No effect of polymorphism	
					-634G>C	C/C decreased grade 3-4 hypertension (0% vs 19%)	No effect of polymorphism	

Table 1. Pharmacogenetic studies in drugs used for colorectal cancer

Differences between genotypes are for homozygote wildtype versus homozygote variant genotype, unless indicated otherwise.
(A)GC: (advanced) gastric cancer; CSS: cancer specific survival; ERCC1: excision repair cross complementation group1; FCGR: Fragment C immunoglobin C receptor; HIF-1α: Hypoxia inducible factor 1α; HNSCC: head and neck squamous cell carcinoma; (m)CRC: (metastasized) colorectal cancer; MST: median survival time; MTHFR: Methylenetetrahydrofolate reductase; NA: not assessed; NS: not specified; NSCLC: non small cell lung carcinoma; OS: overall survival; RR: response rate; TS: thymidylate synthase: TSER: Thymidylate synthase enhancer region; TTP: time to progression; UGT1A1: UTP-glucuronosyltransferase 1A1; VEGF: vascular endothelial growth factor; XPD: xeroderma pigmentosum group D; XPG: xeroderma pigmentosum group G; XRCC1: X-ray cross complementation group1.

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