Combined analysis of genetic polymorphisms in thymidylate synthase, uridine diphosphate glucoronosyltransferase and X-ray cross complementing factor 1 genes as a prognostic factor in advanced colorectal cancer patients treated with 5-fluorouracil plus oxaliplatin or irinotecan

EVA MARTINEZ-BALIBREA, JOSE LUIS MANZANO, ANNA MARTINEZ-CARDUS, TERESA MORAN, BEATRIZ CIRAUQUI, SILVIA CATOT, MIGUEL TARON and ALBERT ABAD

Medical Oncology Service, Institut Catala Oncologia, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain

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Abstract. The aim of this study was to investigate the influence of combining thymidylate synthase (TS), X-ray cross complementing factor 1 (XRCC1) and uridine diphosphate glucoronosyltransferase (UGT1A1*28) polymorphism genotypes in response rate and time to progression (TTP) in metastatic colorectal cancer patients treated with 5-fluorouracil (5-FU) plus irinotecan or oxaliplatin (OXA). PCR, RFLP, allelic discrimination and direct sequencing were performed to elucidate TS, XRCC1 and UGT1A1*28 genotypes in blood from 71 patients. Patients with a number of favourable genotypes (NFG) ≥1 had a lower progression rate and a better TTP than patients with NFG=0 (log-rank $p<0.03$). In the OXA + 5-FU group, patients with the TS 5' single nucleotide polymorphism and/or XRCC1 genotypes favourable to treatment had a better TTP (log-rank $p=0.02$). The TS 5' tandem repeat polymorphism and the NFG were independent prognostic factors in the Cox-based multivariate analysis ($p<0.03$). These results confirm the influence on patient outcome of these genetic polymorphisms and the possibility of studying them together to predict the outcome in first-line treated colorectal cancer patients.

Correspondence to: Dr Albert Abad, Medical Oncology Service, Institut Catala Oncologia, Hospital Universitari Germans Trias i Pujol, Badalona 08916, Barcelona, Spain
E-mail: aabad@ico.scs.es

Abbreviations: TTD, Spanish group for the Treatment of Digestive Tumours; TS, thymidylate synthase; UGT1A1, uridine diphosphate glucoronosyltransferase; XRCC1, X-ray cross complementing factor 1; 5-FU, 5-fluorouracil; TRP, tandem repeat polymorphism; SNP, single nucleotide polymorphism

Key words: thymidylate synthase, uridine diphosphate glucoronosyltransferase, X-ray cross complementing factor 1, genetic polymorphisms, colon cancer, 5-fluorouracil, oxaliplatin, irinotecan

Introduction

Despite the increasing development of novel antitarget agents against tumours such as the ones in colorectal cancer, treatment of this malignancy is still based on chemotherapeutic agents, with 5-fluorouracil (5-FU) plus oxaliplatin (OXA) or irinotecan (CPT11) combinations being most widely used in first- and second-line treatment (1). Although objective response rates in first-line treatment are over 50%, the main cause of treatment failure is either intrinsic or acquired chemoresistance. Genetic polymorphisms, within the gene sequences of proteins related to the mechanisms of action and/or metabolism of chemotherapeutic agents, have been studied in order to simplify the explanation of these complex mechanisms and thus, pharmacogenetics could be an important tool in predicting the outcome of therapy in terms of response and toxicity (2).

5-FU inhibits tumour growth by the covalent binding of its active metabolite, FdUMP, to thymidylate synthase (TS), thereby causing cytotoxicity by dTTP pool depletion, and leading to thymineless death (3). The chronic uracil misincorporation into DNA, which leads to strand breaks initiated by uracil-DNA-glycosylase, and the FUTP misincorporation into RNA, are also part of the 5-FU mechanisms of action, although it has been reported that the latter is predominant when 5-FU is administered as a bolus and not by continuous infusion (4). Several authors have studied the impact of increased TS mRNA levels on treatment outcome. However, a recent metaanalysis shows the necessity for more homogeneous, prospective, and larger studies, in order to determine its real applicability in clinical practice (5). The promoter region of the TS gene is polymorphic [TS 5' tandem repeat polymorphism (TRP)], consisting of either 2 (2R) or 3 (3R) tandem repeats of 28 bp. The triple allele is associated with a higher gene transcription and protein translation and is linked to a poorer response in patients receiving fluoropyrimidine-based treatment (6,7). Some studies have reported the discovery

of a novel G→C single nucleotide polymorphism (TS 5'SNP) in the second repeat of the 3R alleles within a USF consensus element that alters the binding ability of the USF proteins, and thus alters the transcriptional activation of the TS gene bearing this genotype (8). The TS genotypes can be separated into 2 categories: Low (2R/2R, 2R/3RC and 3RC/3RC) and high expression profiles (2R/3RG, 3RC/3RG and 3RG/3RG). Recently, Marcuello *et al* (9) demonstrated an association between the latter and a worse response and survival in patients who had received first line chemotherapy containing 5-FU.

CPT11, after conversion by hCE2 to its active metabolite, SN38, binds to the DNA-Topoisomerase I (Topo I) complex, inhibiting processes such as replication and DNA repair, leading to the apoptosis of tumour cells (10). SN38 is mainly eliminated by hepatic glucuronidation through uridine diphosphate glucoronosyltransferase (UGT1A1), although other mechanisms such as oxidation by CYP3A4 have been related to its inactivation (11,12). Within the UGT1A1 gene promoter region, there is an atypical TATA box consensus element ($A(TA)_nTAA$) which is inherited in a polymorphic way, leading to two major alleles in the Caucasian population consisting of either 6 (6TA) (wild-type alleles) or 7 TA (7TA) repeats (variant alleles). This polymorphism has been named UGT1A1*28. The presence of the 7th repeat results in a 70% reduction in transcriptional activity compared with the wild-type allele. This is the cause of Gilbert's syndrome (13). Patients who are either heterozygous or homozygous for this variant allele exhibit a decreased expression of UGT1A1 and are predisposed to SN38-initiated toxicity in terms of leukopenia and diarrhoea. Some authors have also reported an association between the genotype containing 7 repeats and the overall survival in patients who have received CPT11 plus 5-FU chemotherapy (14).

Oxaliplatin is a third generation diaminocyclohexane (DACH) platinum compound that forms mainly intrastrand links between two adjacent guanine residues or a guanine and an adenine residue, disrupting DNA replication and transcription (15). Although the related platinum compounds, cisplatin and carboplatin, are generally ineffective in the treatment of colorectal cancer, oxaliplatin has been shown to be effective in the treatment of this disease, either in an adjuvant or palliative setting (16,17). Resistance mechanisms to this drug are complex, and are related to the decreased uptake/increased extrusion of the drug, inactivation by glutathione, increased tolerance to DNA adducts, defective capability of DNA repair systems, etc. Indeed, one of the best known mechanisms is that which involves DNA repair systems. The X-ray cross complementing factor 1 (XRCC1) is a scaffolding protein that participates in the base excision repair (BER) pathway. It forms a complex with DNA-ligase III, PARP and DNA polymerase-ß in the final steps of damage removal. It should be noted that, XRCC1-deficient mice do not survive, demonstrating its importance in resealing strand breaks that occur during embryonic development (18). The SNP (Arg>Gln) resides at the C-terminal side of the PARP-interacting domain, within the relatively non-conserved region between the conserved residues of the BRCT domain. This SNP (XRCC1 Arg399Gln) plays a role in protein activity, since several studies have shown a correlation between the variant allele and cancer risk, as well as oxaliplatin-based treatment outcome (19). A link has also been reported between the alterations in the BER pathway and chemoresistance to fluoropyrimidines (20), thus promoting interest in studying this SNP together with others in the TS gene.

Table I. Patient characteristics.

Factor	N (%)
ECOG	
0-1	63 (89)
2	8 (12)
Age (median, range)	60, 33-80
≤50	12 (17)
(50-65)	35 (49)
>65	24 (34)
Gender	
Men	42 (59.2)
Women	29 (40.8)
Primary tumour	
Colon	48 (67.6)
Rectum	23 (32.4)
Metastatic site	
Liver	41 (57.7)
Lung	10 (14.1)
Other	20 (28.2)
Number of metastatic sites	
1	55 (77.5)
>1	16 (22.5)
Adjuvant chemotherapy	
NO	37 (52.1)
5-FU	34 (47.9)
1st line chemotherapy	
OXA + 5-FU	41 (56.3)
CPT11 + 5-FU	20 (26.8)
5-FU	10 (14.1)
Objective response	
Complete	8 (11.3)
Partial	25 (35.2)
Stable disease	26 (36.6)
Progression	12 (16.9)

OXA, oxaliplatin; CPT11, irinotecan; ECOG, performance status.

Some studies have demonstrated the relationship between each of these genetic variants and the outcome of chemotherapy. The aim of this study was to assess these genetic polymorphisms which are related to the main drugs administered simultaneously in first-line treatment, and their capacity to predict response and time to progression (TTP) in a group of non-selected colorectal cancer patients.

Patients and methods

Patients. Seventy-one consecutively observed patients who were diagnosed with metastatic colorectal cancer and with an

Table II. Primers and probes.

Gene	Oligonucleotide sequences 5'→3'
TS	
PCR primers	CGGTCGACCAGACGGTTCCCAAAGGGCG
	GCTCCGAGCCGGCCACAGGCATGGCGCGG
UGT1A1	
PCR primers	CTGAAAGTGAACTCCCTGCTACCT
	CATGGCGCCTTTGCTCCTG
Sequencing primer	Cy5-CCTGCTACCTTTGTGGACTGA
XRCC1	
Allelic discrimination primers	CAGTGGGTGCTGGACTGTCA
	GCAGGGTTGGCGTGTGA
Allelic discrimination probes	(FAM) CCTCCCGGAGGTAA
	(VIC) CCTCCCAGAGGTAA

ECOG performance status of ≤2 were included in the analysis. All the patients had measurable tumour masses for response assessment and they were followed-up to evaluate their response and progression-free survival. Toxicity and objective response were evaluated according to the WHO criteria. The patients received 5-FU-based chemotherapy regimens: i) 5-FU 3.5 g/m^2 continuous infusion (CI) 48-h weekly [Spanish group for the Treatment of Digestive Tumours (TTD) regimen], ii) 5-FU 2.25 g/m^2 CI 48-h weekly plus oxaliplatin 85 mg/m^2 (TTD regimen) or FOLFOX (de Gramont regimen) and iii) 5-FU 2.25 g/m^2 CI 48-h weekly plus CPT11 180 mg/m^2 weekly. The study protocol was approved by the local ethics committee and all the subjects gave informed consent before being included in the study. The patients' characteristics are summarized in Table I. One hundred healthy blood donors (all Caucasians) were also genotyped as the control population in order to assess the Hardy-Weinberg equilibrium in our population.

Genotyping. A total of 71 genotypes (71 TS 5'TRP, 65 TS 5'SNP, 70 XRCC1, and 71 UGT1A1) were determined in the DNA extracted from peripheral blood samples using the QiAmp DNA Blood mini kit (Qiagen) according to the manufacturer's instructions. The TS promoter region was amplified by standard PCR. The 5'TRP alleles were directly analyzed by 3% agarose gel electrophoresis stained with ethidium bromide as the combination of products of 214 (2R) and 242 (3R) bp. The SNP was analyzed by RFLP. Twenty microliters of TS 2R/3R and 3R/3R PCR products was digested with the *HaeIII* restriction enzyme (Invitrogen). The products were loaded onto a 4% LM-SIEVE agarose gel (Conda Laboratories) containing ethidium bromide and electrophoresed. The UGT1A1*28 polymorphism was assessed by direct sequencing as previously described (21). The XRCC1 Arg399Gln genotypes were determined using the 5' nuclease allelic discrimination assay in an ABI PRISM 7000 sequence detection system (Applied Biosystems). This method is based on the PCR technique and uses a set of primers and two probes, each one specifically designed to hybridize with one allele. The PCR conditions were as recommended by the manufacturer. To confirm the accuracy of genotyping with the use of this method, 25 randomly selected DNA samples were subjected to PCR and DNA sequencing. The TS, XRCC1 and UGT1*28 polymorphisms were studied in all the patients and controls, although later, genotypes were considered depending on the chemotherapy regimen. Thus, for patients who received 5-FU + OXA we took into account the TS and XRCC1 genotypes while for patients who received 5-FU + CPT11, we took into account the TS and UGT1A1 genotypes. Objective response and TTP for patients who received 5-FU alone, were correlated with the TS genotypes only. The primers and probes used are listed in Table II.

Statistical analysis. Tumour response and TTP were considered as the end points in this analysis. The overall survival was not calculated since >70% of the patients received 2nd- and 3rd-line therapy and we considered that this fact could mask the effect of the genotype in a global outcome. TTP was calculated from the time that the patient started treatment until disease progression. Patients who stopped treatment or died prior to progression were excluded from this study. Contingency tables and Fisher's exact test were used to evaluate the association of the polymorphisms with either the baseline data or the response to first-line chemotherapy. Kaplan-Meier plots and the log-rank test were used in a univariate analysis to compare the TTP of patients according to the genotype or baseline characteristics. The relative risk ratio and its associated 95% confidence interval were also calculated. The Cox regression method was used for the TTP multivariate analysis. The differences were considered statistically significant when two-sided p-values were $p<0.05$.

Results

Genotypic and allelic frequencies. Genotyping was performed on all the patients and controls in order to evaluate the Hardy Weinberg equilibrium, and also, to rule out any possibility of an association between genetic status and colorectal cancer. No statistically significant differences were found between the observed and expected frequencies in the control

Table III. Genotypic and allelic frequencies.

	Genotypic frequencies					Allelic frequencies		
Gene	wt/wt (%)		wt/var (%)		var/var (%)	wt	var	P[a]
TS 5'TRP								
Controls	37 (37)		43 (43)		20 (20)	0.6	0.4	0.33
Cases	20 (28)		31 (44)		20 (28)	0.5	0.5	
UGT1A1								
Controls	42 (42)		47 (47)		11 (11)	0.7	0.3	0.96
Cases	31 (44)		32 (45)		8 (11)	0.7	0.3	
XRCC1								
Controls	39 (47.6)		32 (39)		11 (13.4)	0.7	0.3	0.57
Cases	30 (42)		33 (47)		7 (10)	0.7	0.3	
	2R/3RC	2R/3RG	3RC/3RC	3RC/3RG	3RG/3RG	3RC	3RG	P[a]
TS 5'SNP								
Controls	8 (17)	10 (21)	3 (6)	7 (15)	3 (6)	0.22	0.24	0.86
Cases	15 (22)	14 (20)	4 (6)	10 (14.5)	4 (6)	0.27	0.23	

wt, wild-type allele; var, variant allele; [a]comparison of the p-value between the control and case genotypes; p-values are based on the Chi-square test. Wild-type alleles are 2R (TS), 6TA (UGT1A1) and Arg (XRCC1).

Table IV. Genotype combinations according to chemotherapy.

Group	Favourable ≥1				Favourable 0		
	Genotypes			N	Genotypes		N
OXA + 5-FU	2/2+Arg/Arg	2/2+Arg/Gln 2/2+Gln/Gln	2/3+Arg/Arg	27[a]-32[b]	2/3+Arg/Gln 2/3+Gln/Gln	3/3+Arg/Gln 3/3+Gln/Gln	14[a]-7[b]
CPT11 + 5-FU	2/2+6/6 2/2+6/7	2/2+7/7	2/3+6/6 3/3+6/6	12[a]-13[b]	2/3+6/7 2/3+7/7	3/3+6/7 3/3+7/7	8[a]-6[b]
5-FU		2/2		0[a]-3[b]	2/3	3/3	10[a]-6[b]

The 'R' in the TS genotypes as well as the 'TA' in UGT1A1 genotypes have been excluded. The TS genotypes correspond either to 5'TRP or 5'SNP. The 5'SNP genotypes are recoded as follows: 2/3C and 3C/3C as 2/2; 2/3G and 3C/3G as 2/3; and 3G/3G as 3/3. [a]Totals for TS 5'TRP and [b]TS 5'SNP.

population, confirming the Hardy Weinberg equilibrium. The genotypic and allelic frequencies are shown in Table III. No statistically significant differences were found in the genotypic frequencies between the patients and the controls.

TS, UGT1A1 and XRCC1 polymorphisms and response to treatment. In order to simplify the analysis, we created a variable that took into account the three studied genes and their relationship with the chemotherapy regimen, thus obtaining two categories: Number of favourable genotypes (NFG) ≥1 or NFG=0 (22). The heterozygous genotypes were grouped into the unfavourable category, and given the sample size, this gave us a more uniform dichotomization. The combinations of genotypes and chemotherapy as well as the number of patients with favourable or unfavourable profiles within each chemotherapy group are shown in Table IV. In the 71 patients analysed, the objective response rate was 46.5%, and 16.9% of the patients had a tumour progression.

Table V. Genotype, baseline characteristics and progression.

Characteristic	No progression (%)	Progression (%)	RR	95% CI	P-value
Gender					
Men	33 (78.6)	9 (21.4)			0.34[a]
Women	26 (89.7)	3 (10.3)			
Primary tumour					
Colon	40 (83.3)	8 (16.7)			1[a]
Rectum	19 (82.6)	4 (17.4)			
1st-line chemotherapy					
OXA + 5-FU	35 (85)	6 (15)			
CPT11 + 5-FU	16 (80)	4 (20)			0.85[b]
5-FU	8 (80)	2 (20)			
Number of metastatic sites					
1	35 (85.4)	6 (14.6)			0.75[a]
>1	24 (80)	6 (20)			
Adjuvant chemotherapy					
NO	31 (83.1)	6 (16.2)			1[a]
5-FU	28 (82.4)	6 (17.6)			
Age					
≤50	9 (75)	3 (25)			
(50-65)	31 (88.6)	4 (11.4)			0.46[b]
>65	19 (79.2)	5 (20.8)			
TS 5'TRP					
2R/2R	20 (100)	0	1	-	0.015[a]
2R/3R or 3R/3R	39 (76.5)	12 (23.5)	1.3	1.12-1.5	
TS 5'SNP					
2R/2R	34 (85)	6 (15)			0.7[a]
2R/3R or 3R/3R	22 (81.5)	5 (18.5)			
[c]TS + UGT1A1 + XRCC1					
Favourable ≥1	36 (92.3)	3 (7.7)	1	-	0.029[a]
Favourable 0	23 (72)	9 (28)	4.7	1.15-19.2	

[a]Fisher's exact test; [b]Chi-square test; [c]TS 5'TRP; RR, relative risk of progression; CI, confidence interval.

The patients with NFG ≥1 (51.3%) responded to therapy (CR or PR) as did 46% of those without favourable genotypes (p=NS). Moreover, the patients with NFG ≥1 had a number of progressions significantly lower than those with NFG of 0 (7.7% vs 28%; p=0.029) which could be indicative of intrinsic resistance to the treatment. It should be noted, that the latter had a 4.7-fold relative risk of progression as compared to the patients with some favourable genotypes. A similar trend was observed when TS 5'SNP was taken into account, although the differences were not statistically significant.

Given that all the regimens were 5-FU based, we analyzed the TS genotypes in the whole group and also found a difference in the number of tumour progressions between the individuals carrying a 3R allele and those whose genotype was 2R/2R. All the progressions occurred within the 3R/2R or 3R/3R group (0% vs 23.5%; p=0.015). This effect was not detected when the patients were separated into high and low TS expression groups, taking into account TS SNP genotypes. No other clinically relevant factors were found to be correlated with response or the number of progressions. The data are summarized in Table V.

*UGT1A1*28- and CPT11-based chemotherapy*. Despite the low number of patients receiving 5-FU plus CPT11 (n=20),

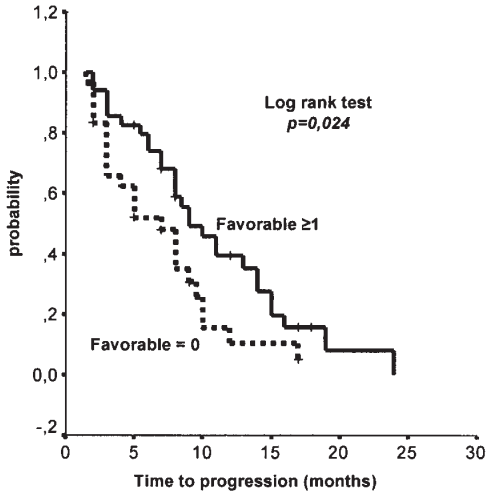

Figure 1. Kaplan-Meier plot for time to progression (TTP) analysis based on the 5'TRP, UGT1A1 and XRCC1 polymorphism genotypes in 65 patients.

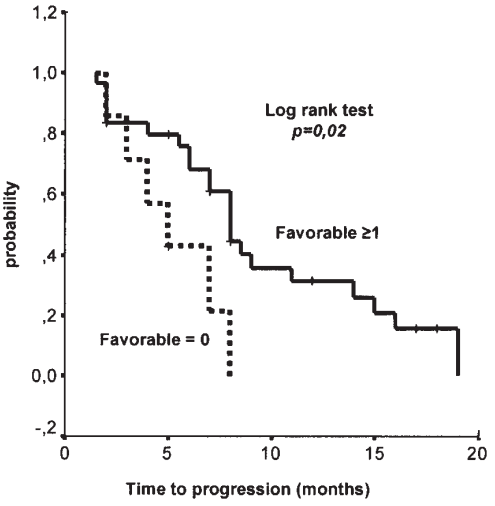

Figure 3. Kaplan-Meier plot for time to progression (TTP) analysis based on the TS 5'SNP and Arg399Gln XRCC1 genotypes in 37 patients treated with oxaliplatin + 5-FU.

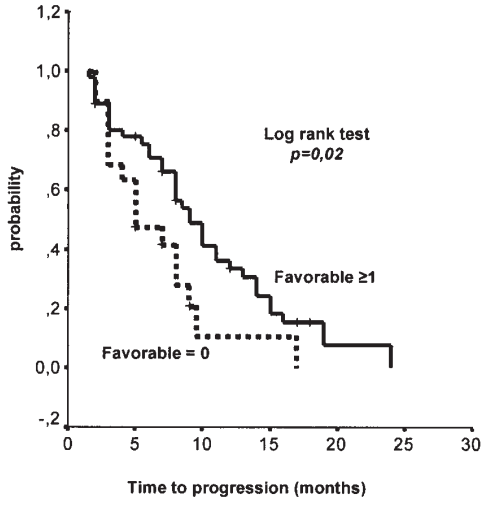

Figure 2. Kaplan-Meier plot for time to progression (TTP) analysis based on the TS 5'SNP, UGT1A1*28 and Arg399Gln XRCC1 genotypes in 65 patients treated with 5-FU plus oxaliplatin or irinotecan.

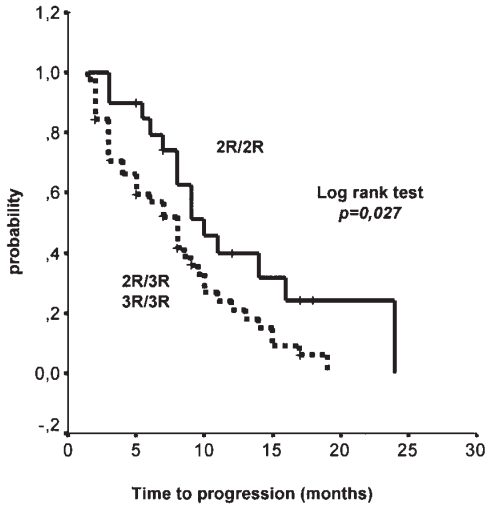

Figure 4. Kaplan-Meier plot for time to progression (TTP) analysis based on the TS 5'TRP genotypes in 65 patients treated with 5-FU-based chemotherapy.

we wanted to investigate the relationship between toxicity and the UGT1A1*28 genotypes in this group of patients. No statistically significant differences were found between the genotypes and neutropenia or diarrhoea. Indeed, patients who were homozygous for the wild-type allele experienced a greater percentage of diarrhoeas compared to those who were heterozygous and homozygous for the variant allele (44.4% vs 20%, p=NS). We also investigated the possible role of the UGT1A1*28 genotypes in response to irinotecan-based chemotherapy. The 6TA/6TA patients (66.7%) responded to treatment while only 40% of the heterozygous or 7TA/7TA patients did so. However, these differences did not reach statistical significance (p=0.37).

TS, UGT1A1 and XRCC1 polymorphisms and TTP. TTP was calculated in months from the beginning of therapy to the time of tumour progression for 65 patients. Twenty percent of the patients had not progressed by the time of the analysis. Patients with NFG ≥1 had a median TTP of 9 months for both TS 5'TRP and TS 5'SNP, while patients who had NFG of 0 had a median TTP of 7 (TS 5'TRP) and 5 (TS 5'SNP) months. Log-rank test p-values were 0.024 and 0.02, respectively. Kaplan-Meier plots for both cases are shown in Figs. 1 and 2. We also investigated the effect of combined genotypes on TTP within the OXA + 5-FU group. Patients with TS (either 5'TRP or 5'SNP) and/or an XRCC1 favourable genotype had a TTP greater than those patients without any of these favourable genotypes (8 months vs 5 months), but differences were statistically significant only for TS 5'SNP (p=0.02) (Fig. 3). In the whole group, only the TS 5'TRP genotypes correlated with TTP individually. Thus, patients with a 2R/2R genotype had a TTP of 10 months compared to patients with a 2R/3R or 3R/3R genotype whose TTP was 8 months (p=0.027) (Fig. 4). As the percentage of responses in the CPT11 plus

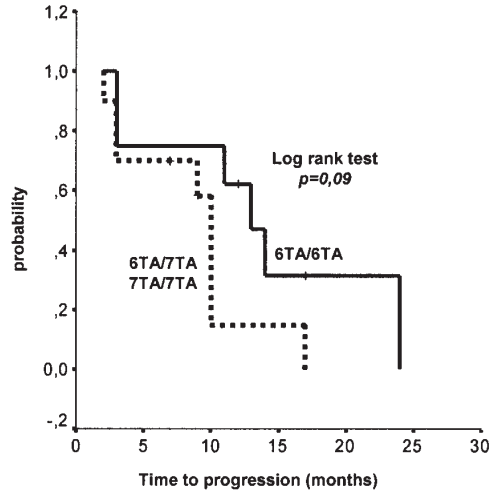

Figure 5. Kaplan-Meier plot for time to progression (TTP) analysis based on the UGT1A1*28 genotypes in 19 patients treated with 5-FU-based chemotherapy.

5-FU group was greater in the 6TA/6TA patients, we wanted to ascertain whether this effect was also observed for TTP. The median TTP for the 6TA/6TA group was 13 months compared to 10 months for patients carrying the 7TA allele (Fig. 5). These differences did not attain significance (p=0.09), probably due to the small number of patients. A summary of the data regarding progression-free survival analysis is presented in Table VI.

Multivariate analysis. Despite the lack of association of any of the clinical characteristics with TTP in the univariate analysis (Table VI), we wanted to include some of them in the multivariate analysis in order to avoid false conclusions. Thus, in the Cox regression analysis, the TS 5'TRP genotypes (p=0.021) as well as the combined genotypes (p=0.027 and p=0.009 for TS 5'TRP and 5'SNP, respectively) were independent prognostic factors of TTP after adjustment to other clinically relevant variables. The data regarding Hazard ratios and 95% confidence intervals are summarized in Table VI.

Discussion

In this study, we investigated why colorectal cancer patients respond differently to first-line treatment combinations of 5-FU plus oxaliplatin or CPT11. To do this, we studied the relationship between the genetic variants in genes whose activity is related to these drugs. Thus, we chose the genetic

Table VI. Genotype, baseline characteristics and TTP.

Variable	RR	95% CI	Median TTP	Log-rank p-value	Cox p-value
Gender					
Men	1.16	(0.66-2.04)	8	0.6	-
Women	1	-	9		
Primary tumour					
Colon	0.91	(0.52-1.62)	8	0.75	-
Rectum	1	-	9		
Number of metastatic sites					
1	0.86	(0.49-1.5)	8	0.6	-
>1	1	-	8.5		
Age					
≤50	0.99	(0.45-2.2)	8		
(50-65)	0.72	(0.4-1.3)	9	0.5	-
>65	1	-	7		
5'TRP					
2R/2R	1	-	10	0.027	0.021
2R/3R+3R/3R	2.2	(1.12-4.14)	8		
TS 5'TRP + UGT1A1 + XRCC1					
Favourable ≥1	1	-	9	0.024	0.027
Favourable 0	1.9	(1.08-3.4)	7		
TS 5'SNP + UGT1A1 + XRCC1					
Favourable ≥1	1	-	9	0.02	0.009
Favourable 0	2.4	(1.2-4.7)	5		

TTP, Time to progression; RR, TTP relative risk; CI, confidence interval. RR and 95% CI values are based on Cox regression analysis. Cox p-values are based on multivariate analysis adjusted for clinical variables.

variants within the TS and UGT1A1 gene promoter sequences and the Arg399Gln XRCC1 polymorphism because of their roles in 5-FU, CPT11 and oxaliplatin action, respectively. Genotyping was performed in the genomic DNA from PBLs by using rapid laboratory techniques which allowed us to obtain results in a few days. This is an important factor in a prospective study. Although some authors have already reported the impact of these genetic variants in these and other related treatments, to our knowledge, this is the first report in which the combination of all of these has been studied and moreover, it has allowed us to distinguish a group of patients with a higher probability of treatment failure.

The discovery of polymorphisms within the TS promoter region as well as their implication in gene transcription and translation (8,23), gives information on TS mRNA and protein levels without the necessity of obtaining tumour samples. In our group of patients, TS 5'TRP was independently associated to response and TTP, demonstrating its predictive and prognostic value in patients treated with 5-FU-based chemotherapy. This is consistent with other studies (6,7). However, the different genotypes of TS 5'SNP did not add any prognostic information, contrary to what we had expected (9). In a recent report, the correlation between the different TS 5'SNP genotypes and intratumoural TS mRNA levels in colorectal cancer patients was studied for the first time (24). Although the study was conducted on a relatively small sample, the authors separated the 3RG/3RC genotype from the others due to its different TS mRNA levels (more similar to those of the low expression group) and consequently obtained statistically significant differences. Indeed, the differences in TTP were greater between the 5'TRP genotypes than between the 5'SNP high and low expression groups. These data are consistent with our results.

In our small subgroup of CPT11-treated patients we could not find any association between the UGT1A1*28 genotypes and toxicity. We observed a trend towards a higher diarrhoea and response rate in the 6TA/6TA patients. Recently, a lack of correlation was reported between the UGT1A1 promoter polymorphism and toxicity to CPT11 in patients treated with capecitabine + irinotecan (25). These authors also observed that patients with the 6TA/6TA genotype had a higher percentage of diarrhoeas than patients carrying the 7TA allele. In another report, an association of the 6TA/6TA genotype was observed with response to CPT11-based first-line chemotherapy (14). A possible explanation for these results resides in the complex pharmacology of irinotecan and the enterohepatic recirculation. Drugs inactivated through glucuronidation in the liver return to the gut once they have left the gall bladder. There, conjugates such as SN38G, are eliminated by microbial ß-galactosidases, and are reconverted into active metabolites. It is possible that this could contribute to the activity of some drugs. Indeed, there is some evidence on the role of these enzymes in CPT11-induced toxicity in the gut, and how this could be reduced by antibiotic administration (26,27). Therefore, a higher glucuronidation in 6TA/6TA patients could promote enterohepatic recirculation leading to: i) Higher drug disposition and ii) higher diarrhoea. Hence, the 7TA/7TA genotype could be predisposed to increased plasma levels of SN38 and increased susceptibility to systemic toxicities such as neutropenia. It should be noted that, UGT1A1 low activity alleles have been more clearly linked to this kind of toxicity rather than diarrhoea (28-30).

The combined analysis of all the genes resulted in a worse response and TTP, according to the chemotherapy regimen, in those patients with unfavourable genotypes who had a 5-fold relative risk of progression compared to those with NFG ≥1. Moreover, in the OXA + 5-FU subgroup, the patients with the TS 5'SNP or XRCC1 favourable genotype had a longer TTP. To our knowledge, this is the first study in which the Arg399Gln XRCC1 and TS genotypes have been analyzed together in order to elucidate their possible role in predicting the outcome to oxaliplatin + 5-FU first-line chemotherapy. These results are in agreement with those reported by Li *et al*, demonstrating a link between the BER system and response to TS inhibitors *in vitro* (20). These authors revealed that a deficiency in ß-polymerase or XRCC1 proteins results in an increased resistance to these kinds of drugs. TS inhibition provokes decreased thymidylate levels, and consequently leads to a nucleotide pool imbalance which leads to uracil misincorporation into DNA strands. Uracil-DNA-glycosylases (UDGs) remove uracil from DNA and promote apoptosis signalling if they are unable to repair it. It has been reported that the overexpression of UDGs leads to an increased resistance to TS inhibitors (31). Hence, an increased TS expression (high expression genotypes) would have an additive effect over the diminished capacity of XRCC1 (Gln/Gln genotype) to promote apoptosis in tumours treated with 5-FU, which could be reflected in a lower response rate and TTP.

Although this analysis has been carried out retrospectively, prospective studies encourage us to continue our investigation in the field of pharmacogenetics (7). We have demonstrated that i) we can separate patients undergoing first-line treatment into two groups with a different clinical outcome based on their genetic characteristics, ii) genetic testing can be performed easily and quickly and iii) this could be translated into a clinical benefit.

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