EUROPEAN JOURNAL OF CANCER 44 (2008) 1229–1237

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Pharmacogenetic approach for capecitabine or 5-fluorouracil selection to be combined with oxaliplatin as first-line chemotherapy in advanced colorectal cancer

Eva Martinez-Balibrea[a], Albert Abad[a,], Enrique Aranda[b], Javier Sastre[c], Jose Luis Manzano[a], Eduardo Díaz-Rubio[c], Auxiliadora Gómez-España[b], Jorge Aparicio[d], Teresa García[e], Inmaculada Maestu[f], Anna Martínez-Cardús[a], Alba Ginés[a], Elisabet Guino[g], on behalf of the Spanish Group for the Treatment of Digestive Tumours (TTD group)*

[a]*Medical Oncology Service, Hospital Universitari Germans Trias i Pujol-Institut Català d'Oncologia, Badalona 08916, Barcelona, Spain*
[b]*Medical Oncology Service, Hospital Reina Sofía, Córdoba, Spain*
[c]*Medical Oncology Service, Hospital Universitario Clínico San Carlos, Madrid, Spain*
[d]*Medical Oncology Service, Hospital Universitari La Fe, Valencia, Spain*
[e]*Medical Oncology Service, Hospital Morales Meseguer, Murcia, Spain*
[f]*Medical Oncology Service, Hospital Virgen de los Lirios, Alicante, Spain*
[g]*Cancer Epidemiology and Statistics Department, Institut Català d'Oncologia–Institut d'Investigació Bellvitge (IDIBELL), L'Hospitalet de LLobregat, Spain*

ARTICLE INFO

Article history:
Received 18 January 2008
Received in revised form 19 March 2008
Accepted 28 March 2008
Available online 28 April 2008

Keywords:
TS 3'UTR
ERCC1-118
Capecitabine
5FU
Oxaliplatin
Polymorphisms
Advanced colorectal cancer

ABSTRACT

We studied the role of *TS* (*5'VNTR*, *5'SNP* and *3'UTR*), *XRCC1-399*, *XPD-751*, *ERCC1-118* and *XRCC3-241* genetic polymorphisms in tailoring fluoropyrimidine/oxaliplatin treatment. For this purpose, 110 XELOX (capecitabine/oxaliplatin)- or FUOX (fluorouracil/oxaliplatin)-treated metastatic colorectal cancer patients were selected prospectively for genotyping. In the FUOX group, *TS-3'UTR* +6 bp/+6 bp (hazards ratio, HR = 2.62, $p = 0.007$) and *ERCC1-118* C/T or C/C (HR = 1.96, $p = 0.050$) genotypes correlated with a shorter progression free survival (PFS). When analysed jointly, the higher the number of favourable genotypes (FG) the longer the PFS (6.8 m, 9.6 m and 25.8 m for 0, 1 or 2 FG; $p = 0.005$). Disease-control rate was 100% in patients with 2 FG (87% and 38.5% for 1 or 0 FG; $p = 0.001$). In the multivariate analysis, *ERCC1-118* (HR = 2.12, $p = 0.0037$) and *TS-3'UTR* (HR = 2.68, $p = 0.006$) were strong independent prognostic factors. According to this, patients harbouring *TS-3'UTR* +6 bp/+6 bp and *ERCC1-118* C/T or C/C genotypes may better receive capecitabine instead of 5FU in an oxaliplatin-based first-line treatment.

* *Corresponding author:* Tel.: +34 497 89 25; fax: +34 497 89 50.
E-mail address: aabad@ico.scs.es (A. Abad).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.03.025

1. Introduction

Oxaliplatin plus 5-fluorouracil (5FU) is one of the standard chemotherapy regimens for first-line treatment in advanced colorectal cancer (CRC) patients. The possible replacement of continuous infusion (CI) of 5FU by the oral pro-drug capecitabine is controversial. Capecitabine has apparent lower toxicity, it is well accepted and avoids the use of portable infusion pumps and catheters. Several controlled phase II and III trials comparing oxaliplatin plus capecitabine (XELOX) or CI of 5FU (FUOX or FOLFOX) have reported similar results concerning response rates (RRs), progression free survival (PFS) and toxicities concluding no differences between both combinations apart from non-haematologic toxicities as hand–foot syndrome which seems to be higher in some of the capecitabine-containing arms.[1–4] These results show therapeutic non-inferiority and similar toxicity but also a cost almost two-fold higher for the capecitabine-containing arms.[5] Therefore, the use of genetic markers to help in the choice of the appropriate fluoropyrimidine would be of clinical interest. Both fluoropyrimidines act mainly through the inhibition of thymidylate synthase (TS), resulting in impaired DNA synthesis and cell death. However, capecitabine needs previous subsequent activation to 5FU by carboxylesterase 2, cytidine deaminase and thymidine phosphorylase (TP).[6] Because TP has high levels of expression in tumours, capecitabine transformation to 5FU preferably occurs at tumour sites.[7] On the other hand, oxaliplatin exerts its action through the formation of DNA and protein adducts which results in DNA replication inhibition and apoptosis.[8] Genetic variability affecting TS and DNA repair genes has been studied as a method to predict the outcome in patients treated with these chemotherapeutic agents. *TS-5'VNTR*, *-5'SNP* and *-3'UTR* polymorphisms have been mainly related to the efficacy of 5FU as a single agent or in combination with other drugs.[9–11] Polymorphisms in *XRCC1-399*, *XPD-751*, *ERCC1-118* and *XRCC3-241* from the base and nucleotide excision repair pathways have shown to modulate the effect of platinated drugs, including oxaliplatin, both *in vitro* and in the clinical setting.[12–15] Specially, *ERCC1-118* variant has been associated with response to oxaliplatin plus 5FU in metastatic colorectal cancer patients.[15] This study was aimed to elucidate the correlation between the efficacy of combined capecitabine/oxaliplatin and 5FU/oxaliplatin and the genetic variations associated with the mechanism of action of these drugs. Furthermore, we aimed to explore the possibility of finding a genetic marker that could allow us to discriminate between both treatments. In the context of current availability of multiple treatment options for patients with advanced CRC, the development of any useful predictive and prognostic pharmacogenomic marker would help in choosing the most appropriate treatment schedule.

2. Materials and methods

2.1. Patients' characteristics and drug administration

This pharmacogenetic analysis was a sub-study of a multicentre, randomised open-label phase III study comparing capecitabine plus oxaliplatin (XELOX) with CI 5FU plus oxaliplatin (FUOX)[16] in patients with metastatic CRC done by the Spanish group for the treatment of digestive tumours (TTD group).[4] Eligible patients were older than 18 years with histologically confirmed metastatic CRC, Karnofsky (PS) $\geqslant 70$ and adequate bone marrow, renal and hepatic functions. Previous cytotoxic chemotherapy was not permitted except for adjuvant treatment completed 12 months before study enrollment. The primary end-point of the pharmacogenetic study was to investigate the association between genotypic data and PFS defined as the time from the start of chemotherapy to the disease progression or death. Response assessments were obtained according to RECIST criteria. Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria, version 2.0 (National Cancer Institute Common Toxicity Criteria, hPFS://ctep.cancer.gov). Patients were randomly assigned to receive either oral capecitabine 1000 mg/m^2 bid for 14 days plus oxaliplatin 130 mg/m^2 on day 1 every 3 weeks or CI of 5FU 2250 mg/m^2 during 48 h on days 1, 8, 15, 22, 29 and 36 plus oxaliplatin 85 mg/m^2 on days 1, 15 and 29 every 6 weeks. Treatment was continued until progressive disease, unacceptable toxicity or consent withdrawal. The pharmacogenetic study protocol was approved by the local ethics committee and all subjects gave informed consent before participating in the study. A total of 110 out of 348 patients consented for prospective genotyping. Table 1 shows a comparison between pharmacogenetic study and full study cohorts. The patients' characteristics are summarised in Table 2.

2.2. Genotyping

Genotypes were determined in DNA extracted from peripheral blood samples using the QiAmp DNA Blood Mini kit (Qiagen) according to the manufacturer's instructions. The TS promoter region polymorphisms (*5'VNTR and 5'SNP*) were analysed through standard PCR and RFLP methods as previously described.[13] The *3'UTR* polymorphism was analysed by GeneScan™ using specific primer sequences: forward 5'-FAM-CAAATCTGAGGGAGCTGAGTAACA-3' and reverse 5'-GAAGGAACTGAGCAGATAAGTGGC-3' in an ABI Prism 3100 DNA Analyzer (Applied Biosystems). The collected data were evaluated with the GeneScan Analysis Software (Applera, Norwalk, CT). Polymorphisms at *XRCC1-399*, *XPD-751*, *ERCC1-118* and *XRCC3-241* were studied by allelic discrimination method in an ABI prism 7000 system. Each PCR was performed following manufacturer's recommendations. Primers and probes for *XRCC1-399* were forward 5'-CAGTGGGTGCT GGACTGTCA-3' and reverse 5'-GCAGGGTTGGCGTGTGA-3'. G-probe 5'-(FAM)-CCTCCCGGAGGTAA and A-probe 5'-(VIC)-CCTCCCAGAGGTAA. For *XPD-751*: forward 5'-GCCTGGAGCA GCTAGAATCAGA-3'; reverse 5'-CACTCAGAGCTGCTGAGCAA TC-3'; T-probe 5'-(FAM)-CTATCCTCTTCAGCGTC-3' and C-probe 5'-(VIC)-TATCCTCTGCAGCGTC-3'. Assays for *ERCC1-118* (C_8901525_10) and *XRCC3-241* (C_2532959_1) were purchased pre-designed by Applied Biosystems.

2.3. Statistical analysis

To detect the differences between genotypes and obtain a HR = 2.1 with $\alpha = 0.05$, 49 patients per arm were sufficient to

Table 1 – Comparison between full study and pharmacogenetic study cohorts

Treatment	Characteristic	Full study N (%)	Sub-study N (%)	p^a
XELOX				
Gender	Male	110 (63.7)	32 (64.3)	0.74
	Female	63 (36.4)	16 (33.3)	
Karnofsky	⩽80	65 (38)	11 (23)	0.06
	>80	108 (62)	37 (77)	
Tumour	Colon	110 (64)	30 (63)	0.62
	Rectum	49 (28)	16 (33)	
	Both	13 (8)	2 (4)	
Metastatic sites	1	106 (64)	35 (73)	0.3
	>1	60 (36)	13 (27)	
Previous ACT	5FU	41 (24)	13 (27)	0.7
	NO	132 (76)	35 (73)	
Objective response	CR + PR	63 (43)	23 (56)	0.2
	SD + PD	83 (57)	18 (44)	
PFS: median (95% CI)		8.78 (7.83–9.74)	11.18 (8.76–13.61)	0.2
FUOX				
Gender	Male	102 (60)	28 (60)	1
	Female	69 (40)	19 (40)	
Karnofsky	⩽80	68 (39)	14 (29)	0.24
	>80	106 (61)	34 (71)	
Tumour	Colon	119 (68.4)	35 (73)	0.8
	Rectum	49 (28.2)	12 (25)	
	Both	6 (3.4)	1 (2)	
Metastatic sites	1	120 (73)	31 (65)	0.3
	>1	45 (27)	17 (35)	
Previous ACT	5FU	24 (14)	13 (27)	0.6
	NO	150 (86)	35 (73)	
Objective response	CR + PR	73 (49)	21 (47)	0.9
	SD + PD	75 (51)	24 (53)	
PFS: median (95% CI)		9.64 (8.33–10.94)	9.41 (7.9–10.9)	0.6

FUOX = 5-fluorouracil + oxaliplatin; XELOX = capecitabine + oxaliplatin; ACT = adjuvant chemotherapy; CR = complete response; PR = partial response; SD = stable disease; PD = progression; PFS = progression free survival; CI = confidence interval.
a *p*-values are based on χ^2 test except for PFS. In that case, *p*-values are based on a log-rank test.

reach a statistical power of 83%. The associations between clinical parameters and the polymorphisms status were assessed considering chemotherapy regimens jointly and independently by using the SPSS 12.0 statistical package. Genotypes for each polymorphism were analysed as a three-group categorical variable (codominant model), and they were also grouped according to the dominant and recessive model. Contingency tables, χ^2 and Fisher's exact test were used to evaluate the association of polymorphisms with categorical variables. Genetic variations were analysed independently unless multiple genotypes showed a significant association in which case we performed a combined analysis. The association of genetic variants and clinical parameters with PFS was estimated by calculating HR and their 95% CI from univariate and multivariate Cox proportional hazards regression models. Kaplan–Meier plots and the log-rank test were used to estimate PFS curves. Each polymorphism was tested to ensure it fitted Hardy–Weinberg equilibrium. Linkage disequilibrium between polymorphisms was calculated by using the SNPstats web tool from the Catalan Institute of Oncology (hPFS://bioinfo.iconcologia.net/index.php?module=Snpstats). Haplotypes were analysed using the haplo.stats package, which implements the expectation maximisation algorithm to estimate the haplotype frequencies. For each individual, the compatible haplotypes and their posterior probabilities were computed and coded with dummy indicator variables. The posterior probabilities were used as weights in the Cox models to account for uncertainty in the identification of phase-unknown haplotypes. The differences were considered statistically significant when two-sided *p*-values were less than 0.05.

3. Results

Of the 110 Caucasian, eligible patients, 96 were assessable for PFS and toxicity and 87 for response (Table 2). The 14 remaining patients were excluded (i) because of response and PFS data were not assessable ($n = 5$) or (ii) sample problems such as blood lysis or inappropriate storage ($n = 9$). A total of 49 patients (51%) received a further chemotherapy treatment when disease progression was documented. Median PFS was 10.3 months (XELOX 11.8; FUOX 9.4) and median overall survival (OS) was 20.8 months (XELOX 20.8; FUOX 18.2); the distribution of the best objective response was as follows: 8.3% had complete response, 38.5% had partial response, 26% had stable disease and 17.7% had tumour progression. Main grade 3–4 toxicities in the total population were paraesthesia (21.8%), diarrhoea (16.7%), neutropenia (9.3%) and vomiting (7.3%).

Table 2 – Demographics and overall response of study subjects

Characteristics	XELOX (%)	FUOX (%)	Total	R[a]	PFS[b]
Subjects	47 (49)	49 (51)	96		
Assessable for genotyping	47 (49)	49 (51)	96		
Assessable for response	41 (47)	46 (53)	87		
Assessable for toxicity	47 (49)	49 (51)	96		
Assessable for PFS	47 (49)	49 (51)	96		
Median age (range), y[c]	62.8 (46.3–78.3)	63.2 (35.9–81.6)	63 (35.9–81.6)	0.3	0.17
Gender					
Man	31 (66)	29 (59.2)	60 (62.5)	0.66	0.9
Woman	16 (34)	20 (40.8)	36 (37.5)		
Karnofsky PS					
100	21 (44.7)	19 (38.8)	40 (41.7)	0.4	0.26
90	15 (30.6)	15 (30.6)	30 (31.2)		
80	7 (14.9)	13 (26.5)	20 (20.8)		
70	4 (8.5)	2 (4.1)	6 (6.3)		
Number of metastatic sites					
1	33 (70.2)	31 (63.3)	64 (66.7)	0.9	0.46
>1	14 (29.8)	18 (36.8)	32 (33.3)		
Previous adjuvant CT					
Yes	12 (25.5)	8 (16.3)	20 (20.8)	0.3	0.8
No	35 (74.5)	41 (83.7)	76 (79.2)		
Primary tumour					
Colon	30 (63.8)	35 (71.4)	65 (67.7)	0.8	0.7
Rectum	15 (31.9)	13 (26.5)	28 (29.2)		
Both	2 (4.3)	1 (2)	3 (3.1)		
Biochemical parameters					
LDH > UNL	27 (71.1)	30 (68.2)	57 (59.4)	0.2	0.6
AP > UNL	25 (54.3)	28 (59.6)	53 (55.2)	0.5	0.3
WBC count > 10×10^9/L	10 (21.3)	15 (30.6)	25 (26)	0.7	0.34
Objective response					
Complete	5 (12.2)	3 (6.5)	8 (9.2)		
Partial	19 (46.3)	18 (39)	37 (42.5)		
Stable disease	11 (26.8)	14 (30.4)	25 (28.7)		
Progression	6 (14.6)	11 (23.9)	17 (19.5)		

XELOX = capecitabine + oxaliplatin; FUOX = 5-fluorouracil + oxaliplatin; R = response; PFS = progression free survival; PS = performance status; CT = chemotherapy; LDH = lactate dehydrogenase; AP = alkaline phosphatase; WBC = white blood cell; UNL = upper normal limit value.
a p-values are based on a χ^2 test.
b p-values are based on a log-rank test.
c Patients were grouped as younger or older than 60 years.

3.1. *Allelic, genotypic and haplotypic frequencies and Hardy–Weinberg equilibrium*

In 96 genotyped patients, allelic and genotypic frequencies were found to be similar to those described in previous reports in white populations (Table 3). Allelic distribution was in Hardy–Weinberg equilibrium. There was no statistical association between clinical baseline characteristics and response or PFS (Table 2). *TS-5'VNTR* and *TS-3'UTR* polymorphisms were in linkage disequilibrium (D' = 0.58; R = –0.32; $p < 0.001$). Estimated haplotypes frequencies are shown in Table 4.

3.2. *Genotypes, haplotypes and clinical outcomes*

In the whole group, no statistical associations were found between PFS and any of the polymorphisms studied. However, a significant shorter PFS was associated with *TS-3'UTR* +6 bp/+6 bp genotype ($p = 0.02$) in the FUOX group (median PFS = 6.4 months (m) versus 14 and 10 m for +6 bp/–6 bp and –6 bp/–6 bp, respectively) (Table 4). In the dominant model, these differences were increased with a HR = 2.62 and 95% CI (1.3–5.3) for the +6 bp/+6 bp genotype ($p = 0.007$). Kaplan–Meier plots for the dominant model are shown in Fig. 1A. As *TS-5'VNTR* and *3'UTR* polymorphisms were found to be in linkage disequilibrium, we analysed the influence of the resultant haplotypes on PFS. Haplotype 2 R+6 bp was associated with a shorter PFS in the FUOX group, but the increased hazard ratio (HR) when compared to 2R–6 bp was probably due to the presence of the +6 bp allele (Table 4). For this reason, we decided to study *TS 3'UTR* genotypes independently. In this group of patients, we also found a trend to a shorter PFS for *ERCC1*-118 C/T or C/C genotypes in the dominant model (HR = 1.96, 95% CI (0.99–3.92) $p = 0.0501$, Table 4). Among all clinical variables, age and Karnofsky showed the lowest p-values for the association with PFS (Table 2) and were included in the multivariate analysis. Interestingly, *ERCC1*-118 C/T or

Table 3 – Genotypic and allelic frequencies

Gene	Allelic frequency	Genotypic frequency, N (%)
TS-5'VNTR		
2R	0.38	2R/2R 20 (21)
3R	0.62	2R/3R 33 (34)
		3R/3R 43 (45)
TS-5'SNP		
3G	0.27	2/3C 17 (18)
		2/3G 15 (16)
3C	0.35	3C/3C 13 (16)
		3C/3G 22 (23)
		3G/3G 7 (7)
TS-3'UTR		
+6 bp	0.67	+6 bp/+6 bp 47 (49)
–6 bp	0.33	+6 bp/–6 bp 35 (36)
		–6 bp/–6 bp 14 (15)
XRCC1-399		
Arg	0.63	Arg/Arg 40 (42)
Gln	0.37	Arg/Gln 39 (41)
		Gln/Gln 16 (17)
XPD-751		
Lys	0.65	Lys/Lys 42 (44)
Gln	0.35	Lys/Gln 40 (42)
		Gln/Gln 14 (15)
XRCC3-241		
Met	0.37	Met/Met 41 (43.2)
Thr	0.63	Met/Thr 38 (40)
		Thr/Thr 16 (16.8)
ERCC1-118		
C	0.39	C/C 17 (18)
T	0.61	C/T 40 (42)
		T/T 39 (41)

C/C genotypes and *TS-3'UTR* +6 bp/+6 bp were the only independent prognostic factors in the FUOX group (Table 5A). Indeed, statistical differences became higher for these genetic variants.

3.3. *Combination of genotypes, PFS and disease-control rate*

Based on individual results for *TS 3'UTR* and *ERCC1-118*, we wanted to investigate the possible interaction between both polymorphisms by dividing the sample into three groups of combining genotypes in such a way that one patient had 2 (group 1, 22% of patients), 1 (group 2, 47% of patients) or 0 (group 3, 31% of patients) favourable genotypes (+6 bp/–6 bp or –6 bp/–6 bp and T/T). As shown in Fig. 1B, the higher the number of favourable genotypes, the longer the PFS with a median of 6.8 months for group 3, 9.6 months for group 2 and 25.8 months for having both TS and ERCC1 favourable genotypes ($p = 0.005$, group 2 HR = 2.57, 95% CI 0.94–7; group 3 HR = 5.63, 95% CI 1.92–16.53). In the multivariate analysis, group 3 was the strongest independent prognostic factor (Table 5B). Again, no differences were found in the XELOX group (Fig. 1C). We tested the predictive value or the identified markers (*ERCC1-118* and *TS-3'UTR*) using a multivariate model with an interaction term = number of favourable genotypes * chemotherapy. As is shown in Table 6, the advantage/disadvantage of FUOX or XELOX is significantly affected by the marker. Thus, a patient with two favourable genotypes receiving XELOX has a HR = 1 and a PFS of 10.5 months, while the same genotype receiving FUOX has a HR = 0.34 and a median PFS of 25.8 months. The opposite happens when a patient has 0 favourable genotypes and if the patient has 1 favourable genotype, there is almost no differences between treatments.

We did not find any association between single polymorphisms and response either in the whole group or in each group of treatment (data not shown). We further investigated the possible association of groups 1, 2 and 3 with progression disease (disease-control rate, DCR). A statistically significant association was found between the number of unfavourable genotypes and progression rate in the FUOX group, with 0 out of 10 progressions in group 1 (DCR 100%), three out of 23 in group 2 (DCR 87%) and 8 out of 13 progressions in group 3 (DCR 38.5%) ($p = 0.001$).

4. Discussion

In this prospective study, a strong association between *TS-3'UTR* and *ERCC1-118* polymorphisms (separately and combined) and PFS as well as disease-control rate was found in colorectal cancer patients treated with Fluorouracil plus oxaliplatin but not in those treated with capecitabine plus oxaliplatin as first-line chemotherapy. Indeed, patients in the FUOX group harbouring two unfavourable genotypes had median PFS shorter than those in the XELOX group (6.8 months versus 11.2) indicating a benefit for these patients to receive capecitabine instead of 5FU. These results suggest different behaviour at the molecular level for the combination of both fluoropyrimidines with oxaliplatin and supporting the idea of a genetic marker to select the most appropriate fluroropyrimidine to be combined with this platinum agent.

Thymidylate synthase polymorphisms located in 5'UTR region are, probably, of the most broadly studied genetic variants in the field of pharmacogenetics in CRC.[17] It is quite accepted that 3R and 3RG alleles are associated with increased levels of the chemotherapeutic target TS (mRNA, protein or activity depending on the different studies)[18–20] and this fact has been associated with poorer outcome in patients treated with 5FU-containing regimens both in the adjuvant and in the metastatic setting.[10,11,21] But some researchers have observed just the opposite which shows up a gap between *in vitro* experiments and clinical findings.[9,22] This could be explained by the presence of other drugs in combination with 5FU in most of these clinical studies, the complexity of tumour environment, the way 5FU is administered (CI or Bolus) or additional variants within this gene region as, for example the recently discovered G to C change in the first repeat of the 2R allele.[23] Our results showed no association between these genetic variants and fluroropyrimidine plus oxaliplatin combination. However, we did find a statistical association between genotypes corresponding to TS-3'UTR polymorphism and clinical outcome in patients of the FUOX group. The 6 bp deletion in the 3'UTR of TS gene has been correlated with increased message instability and consequently, lower levels of TS mRNA.[24] On the other hand, it seems clear that high tumour levels of TS mRNA predict

Table 4 – Univariate Cox proportional hazards regression models for the association of Genetic variants and PFS by treatment

Genotypes[a]	XELOX				FUOX			
	N (%)[d]	HR	95% CI	*p*	*N* (%)[d]	HR	95% CI	*p*
TS-5'VNTR								
2R/2R	9 (19)		1	0.95	11 (22)		1	0.97
2R/3R	16 (34)	0.97	0.34–2.81		17 (35)	0.9	0.36–2.27	
3R/3R	22 (47)	0.88	0.32–2.4		21 (43)	0.94	0.4–2.2	
TS-5'SNP[b]								
Low expression	26 (56)		1	0.76	24 (50)		1	0.64
Intermediate	16 (35)	0.77	0.37–1.6		21 (44)	1.21	0.61–2.38	
High	4 (9)	0.79	0.27–2.35		3 (6)	0.54	0.07–4.07	
TS-3'UTR								
+6 bp/+6 bp	22 (47)		1	0.76	25 (51)		1	0.02
–6 bp/+6 bp	21 (45)	1.21	0.59–2.46		14 (29)	0.32	0.14–0.74	0.008
–6 bp/–6 bp	4 (8)	1.46	0.48–4.41		10 (20)	0.49	0.2–1.19	
XRCC1-399								
Arg/Arg	19 (40.4)		1	0.64	21 (43.8)		1	0.9
Arg/Gln	19 (40.4)	0.82	0.39–1.71		20 (42)	0.85	0.42–1.73	
Gln/Gln	9 (19.1)	0.65	0.25–1.66		7 (14.6)	0.96	0.31–3	
XPD-751								
Lys/Lys	20 (42.6)		1	0.39	22 (43)		1	0.61
Lys/Gln	21 (44.7)	1.05	0.51–2.15		19 (39)	0.94	0.45–1.97	
Gln/Gln	6 (12.8)	0.54	0.19–1.52		8 (18)	1.5	0.61–3.69	
ERCC1-118[c]								
T/T	18 (38.3)		1	0.735	21 (43)		1	0.050
C/T or C/C	29 (61.7)	1.13	0.57–2.24		28 (57)	1.96	0.99–3.92	
XRCC3-241								
Thr/Thr	23 (49)		1	0.48	18 (37.5)		1	0.8
Thr/Met	18 (38)	0.91	0.43–1.9		20 (41.7)	1.22	0.58–2.6	
Met/Met	6 (13)	1.8	0.66–4.95		10 (20.8)	1.33	0.54–3.29	
TS Haplotypes								
2R – 6 bp	(7.5)	1		0.67	(11)		1	0.08
2R + 6 bp	(30)	1.47	0.26–8.29		(30)	2.97	1.06–8.32	0.04
3R – 6 bp	(25.5)	1.58	0.28–8.86		(25)	1.78	0.63–5.04	
3R + 6 bp	(37)	1.15	0.21–6.39		(34)	2.55	0.94–6.95	

p-values correspond to the association test in the cox model; significant *p*-values for individual genotypes are also shown. HR = hazard ratio; CI = confidence interval; XELOX = capecitabine + oxaliplatin; FUOX = 5-fluorouracil + oxaliplatin.

a Codominant models.

b Low expression = 2/2 or 2/3C or 3C/3C; intermediate = 2/3G or 3C/3G; high = 3G/3G.

c Dominant model.

d Values shown for TS haplotypes correspond to estimated frequencies.

for a worse clinical outcome in patients treated with 5FU-based chemotherapy.[25] Thus, patients carrying the +6 bp allele would have a worse prognostic which is confirmed by our results and those from other authors.[9] As had been previously described[9,26], *TS-5'VNTR* and *-3'UTR* polymorphisms were in linkage disequilibrium in our cohort of patients and the 2R/+6 bp haplotype was associated with a shorter PFS in the FUOX group. However, it can be assumed that higher HRs observed for 2R/+6 bp and 3R/+6 bp as compared with 2R/–6 bp and 3R/–6 bp, respectively, are due to the presence of the +6 bp allele (Table 4).

Patients under study were also treated with oxaliplatin, so we wanted to analyse genetic variations affecting its activity. Amongst all studied polymorphisms within genes encoding for DNA repair proteins, *ERCC1-118* was the only one associated with PFS in the FUOX group. C/T and C/C genotypes were associated with shorter PFS time. This is in agreement with a report from Viguier and collegues[15], as they found that patients treated with oxaliplatin plus 5FU chemotherapy carrying the T/T genotype were more likely to respond than those patients carrying one or two C alleles. It is not clear how the C to T change in the gene sequence affects the repair activity of ERCC1 protein but it seems that the substitution of a codon of common usage (AAC) for one of approximate 50% reduced usage (AAT) affects translation efficiency and consequently, protein levels and activity.[27] Impaired repair of oxaliplatin adducts would result in increased cytotoxic activity. *In vitro* experiments have demonstrated that previous administration of this platinated drug can reduce TS levels thus facilitating 5FU action.[28] In the FUOX group there was an interaction between TS and ERCC1 polymorphisms as in the multivariate analysis, statistical differences between geno-

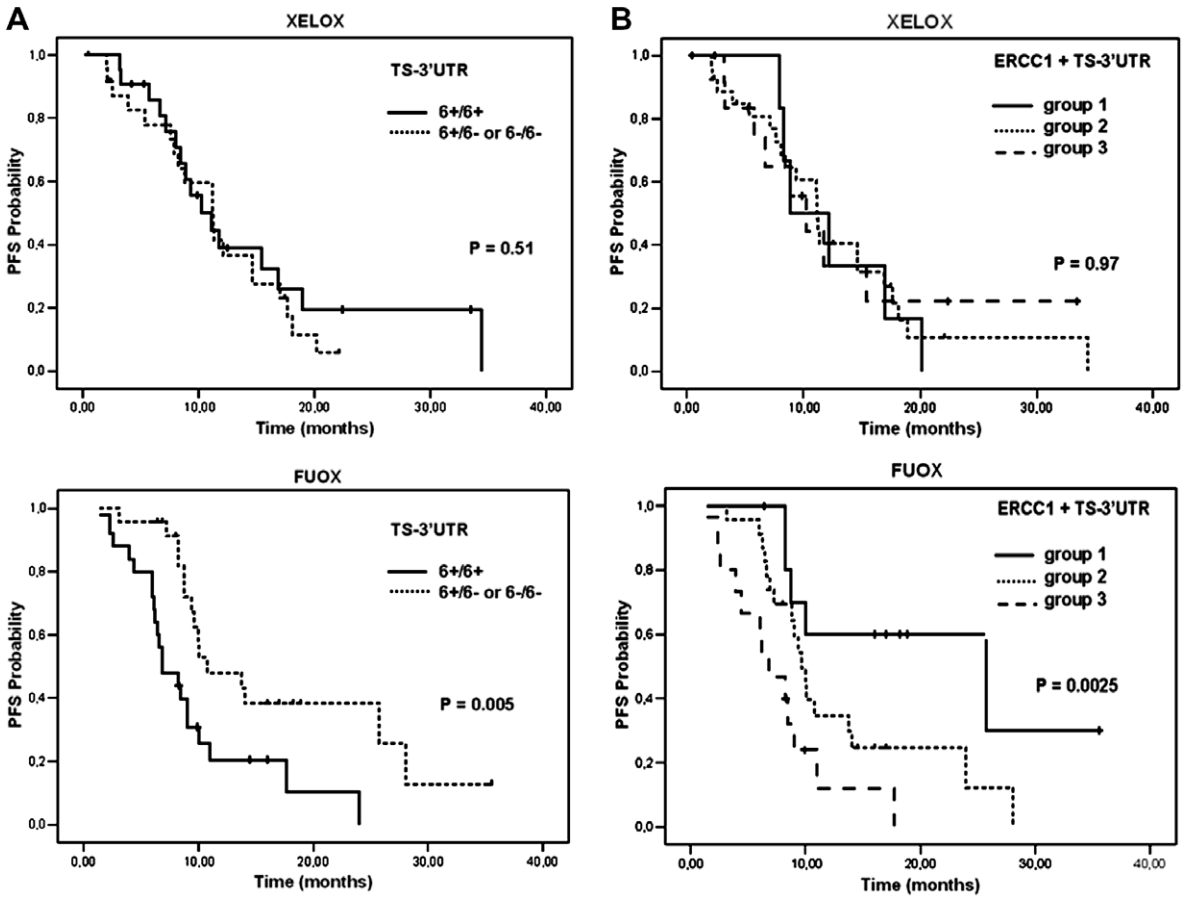

Fig. 1 – PFS Kaplan–Meier plots for PFS according to (A) TS-3'UTR and (B) the combination of ERCC1-118 and TS-3'UTR genotypes in XELOX and FUOX groups of treatment. *p*-Values correspond to the log-rank test.

Table 5 – Multivariate analysis for FUOX

Factor	HR	95% CI	*p*
(A) TS-3'UTR and ERCC1-118 genotypes and PFS			
ERCC1-118 T/C or C/C	2.12	1.05–4.28	0.037
TS-3'UTR +6 bp/+6 bp	2.68	1.33–5.43	0.006
Age < 60 y[a]	1.32	0.67–2.61	0.425
Karnofsky PS 70 or 80	0.61	0.3–1.25	0.177
(B) TS and ERCC1 combined genotypes and PFS			
1 Favourable genotype	2.62	0.95–7.18	0.062
0 Favourable genotypes	5.9	1.98–17.52	0.001
Age <60 y[a]	1.3	0.66–2.57	0.45
Karnofsky PS 70 or 80	0.6	0.29–1.22	0.16

FUOX = 5-fluorouracil + oxaliplatin.
a Years; HR = hazard ratio; CI = confidence interval.

types increased. On the other hand, the lack of correlation between these polymorphisms and clinical outcome in the XELOX group could be explained by the fact that capecitabine needs previous subsequent activation to 5FU by carboxylestarase 2, cytidine deaminase and thymidine phosphorylase.[6] Putative alterations in the expression of these genes could affect the final tumour concentration of the active metabolite of capecitabine in the XELOX group, somehow diluting the effect of TS genetic variants (and also ERCC1 in our cohort of patients), whilst the same alterations in the FUOX group would not have the same effect. Indeed, other authors have reported no association between TS gene polymorphisms and oral fluoropyrimidines-based treatment.[29,30] This hypothesis would be supported by the fact that positive TP protein expression correlates with tumour response to capecitabine plus irinotecan[31] and, on the other hand, low tumour levels of TP mRNA are associated with response to 5FU treatment in colorectal cancer patients.[32] Thus, high levels of TP and TS in a patient treated with capecitabine would result in high levels of intratumoural 5FU and consequently, appropriate inhibition of TS. The same characteristics in a patient treated with 5FU would result in a low TS inhibition as 5FU is not metabolised by TP. Further studies at the mRNA and protein levels are warranted to validate this hypothesis.

In this prospective study, we show how a pharmacogenetic approach could help in capecitabine or 5FU selection to be combined with oxaliplatin in first-line chemotherapy in colorectal cancer patients. Although study limitations such as sample size make us confirm these results in larger studies, one can hypothesize that a patient with advanced CRC who carries *TS-3'UTR* +6 bp/+6 bp and *ERCC1*-118 C/T or C/C genotypes may better receive capecitabine instead of 5FU in an oxaliplatin-based first-line treatment. Although prior objective of treatment selection is patients' benefit, the administration of capecitabine has some aspects to have into account,

Table 6 – Interaction between genomic markers and chemotherapy

Interaction term	N	Median PFS (months)	HR	95% CI	p
2 FG*XELOX	8	10.5	1	(0.36–2.18)	0.0186
1 FG*XELOX	27	11.2	0.88	(0.27–2.29)	
0 FG*XELOX	12	10.2	0.79	(0.1–2.29)	
2 FG*FUOX	11	25.8	0.34	(0.1–1.11)	
1 FG*FUOX	23	9.6	0.97	(0.38–2.46)	
0 FG*FUOX	15	6.8	2.1	(0.79–5.57)	

FG = favourable genotypes; PFS = progression free survival; HR = hazard ratio; CI = confidence interval.

such as comparative tolerability, patient convenience and cost. The estimated cost for 12 weeks of treatment with FUOX for a patient with a body surface area of 1.8 m^2 is $27,156, whilst a XELOX treatment in the same conditions has a cost of $50,228.[5] If our results are confirmed, this cost would be justified in those patients with unfavourable genotypes for FUOX since the estimated cost for *TS-3'-UTR* and *ERCC1-118* genotyping based on our own calculations would be less than 12 € per patient. Recent results from the Spanish Lung Cancer Group (SLCG) demonstrate that assigning cisplatin based on pretreatment tumour ERCC1 mRNA levels improves response rate and that a study of these characteristics is possible.[33] Our results and those from other authors merit the conduction of well designed randomised studies comparing the standard treatments selected on a clinical basis with experimental arms selected on the basis of a pharmacogenomic signature.

Conflict of interest statement

None declared.

Acknowledgements

We thank Drs. Josep Tabernero and Noemí Reguart for critical reading of the manuscript.

REFERENCES

1. Braun AH, Achterrath W, Wilke H, Vanhoefer U, Harstrick A, Preusser P. New systemic frontline treatment for metastatic colorectal carcinoma. *Cancer* 2004;**100**(8):1558–77.
2. Schmoll HJ, Arnold D. Update on capecitabine in colorectal cancer. *Oncologist* 2006;**11**(9):1003–9.
3. Porschen R, Arkenau HT, Kubicka S, et al. Phase III study of capecitabine plus oxaliplatin compared with fluorouracil and leucovorin plus oxaliplatin in metastatic colorectal cancer: a final report of the AIO Colorectal Study Group. *J Clin Oncol* 2007;**25**(27):4217–23.
4. Diaz-Rubio E, Tabernero J, Gomez-Espana A, et al. Phase III study of capecitabine plus oxaliplatin compared with continuous-infusion fluorouracil plus oxaliplatin as first-line therapy in metastatic colorectal cancer: final report of the Spanish Cooperative Group for the Treatment of Digestive Tumours Trial. *J Clin Oncol* 2007;**25**(27):4224–30.
5. Mayer RJ. Should capecitabine replace infusional fluorouracil and leucovorin when combined with oxaliplatin in metastatic colorectal cancer? *J Clin Oncol* 2007;**25**(27):4165–7.
6. Quinney SK, Sanghani SP, Davis WI, et al. Hydrolysis of capecitabine to 5'-deoxy-5-fluorocytidine by human carboxylesterases and inhibition by loperamide. *J Pharmacol Exp Ther* 2005;**313**(3):1011–6.
7. Takebayashi Y, Yamada K, Miyadera K, et al. The activity and expression of thymidine phosphorylase in human solid tumours. *Eur J Cancer* 1996;**32A**(7):1227–32.
8. Raymond E, Faivre S, Chaney S, Woynarowski J, Cvitkovic E. Cellular and molecular pharmacology of oxaliplatin. *Mol Cancer Ther* 2002;**1**(3):227–35.
9. Dotor E, Cuatrecases M, Martinez-Iniesta M, et al. Tumour thymidylate synthase 1494del6 genotype as a prognostic factor in colorectal cancer patients receiving fluorouracil-based adjuvant treatment. *J Clin Oncol* 2006;**24**(10):1603–11.
10. Marcuello E, Altes A, del Rio E, Cesar A, Menoyo A, Baiget M. Single nucleotide polymorphism in the 5' tandem repeat sequences of thymidylate synthase gene predicts for response to fluorouracil-based chemotherapy in advanced colorectal cancer patients. *Int J Cancer* 2004;**112**(5):733–7.
11. Pullarkat ST, Stoehlmacher J, Ghaderi V, et al. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogen J* 2001;**1**(1):65–70.
12. de las Penas R, Sanchez-Ronco M, Alberola V, et al. Polymorphisms in DNA repair genes modulate survival in cisplatin/gemcitabine-treated non-small-cell lung cancer patients. *Ann Oncol* 2006;**17**(4):668–75.
13. Martinez-Balibrea E, Manzano JL, Martinez-Cardus A, et al. 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. *Oncol Rep* 2007;**17**(3):637–45.
14. Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei DD, Groshen S, Lenz HJ. A Xeroderma pigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer. *Cancer Res* 2001;**61**(24):8654–8.
15. Viguier J, Boige V, Miquel C, et al. ERCC1 codon 118 polymorphism is a predictive factor for the tumour response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. *Clin Cancer Res* 2005;**11**(17):6212–7.
16. Abad A, Carrato A, Navarro M, et al. Two consecutive phase II trials of biweekly oxaliplatin plus weekly 48-hour continuous infusion of nonmodulated high-dose 5-fluorouracil as first-line treatment for advanced colorectal cancer. *Clin Colorectal Cancer* 2005;**4**(6):384–9.
17. Marsh S. Thymidylate synthase pharmacogenetics. *Invest New Drugs* 2005;**23**(6):533–7.
18. Etienne MC, Chazal M, Laurent-Puig P, et al. Prognostic value of tumoural thymidylate synthase and p53 in metastatic colorectal cancer patients receiving fluorouracil-based chemotherapy: phenotypic and genotypic analyses. *J Clin Oncol* 2002;**20**(12):2832–43.

19. Kawakami K, Salonga D, Park JM, et al. Different lengths of a polymorphic repeat sequence in the thymidylate synthase gene affect translational efficiency but not its gene expression. *Clin Cancer Res* 2001;**7**(12):4096–101.
20. Ishida Y, Kawakami K, Tanaka Y, Kanehira E, Omura K, Watanabe G. Association of thymidylate synthase gene polymorphism with its mRNA and protein expression and with prognosis in gastric cancer. *Anticancer Res* 2002;**22**(5):2805–9.
21. Iacopetta B, Grieu F, Joseph D, Elsaleh H. A polymorphism in the enhancer region of the thymidylate synthase promoter influences the survival of colorectal cancer patients treated with 5-fluorouracil. *Br J Cancer* 2001;**85**(6):827–30.
22. Jakobsen A, Nielsen JN, Gyldenkerne N, Lindeberg J. Thymidylate synthase and methylenetetrahydrofolate reductase gene polymorphism in normal tissue as predictors of fluorouracil sensitivity. *J Clin Oncol* 2005;**23**(7):1365–9.
23. Lincz LF, Scorgie FE, Garg MB, Ackland SP. Identification of a novel single nucleotide polymorphism in the first tandem repeat sequence of the thymidylate synthase 2R allele. *Int J Cancer* 2007;**120**(9):1930–4.
24. Mandola MV, Stoehlmacher J, Zhang W, et al. A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoural TS mRNA levels. *Pharmacogenetics* 2004;**14**(5):319–27.
25. Popat S, Matakidou A, Houlston RS. Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. *J Clin Oncol* 2004;**22**(3):529–36.
26. Ulrich CM, Bigler J, Bostick R, Fosdick L, Potter JD. Thymidylate synthase promoter polymorphism, interaction with folate intake, and risk of colorectal adenomas. *Cancer Res* 2002;**62**(12):3361–4.
27. Yu JJ, Lee KB, Mu C, et al. Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are equally resistant to platinum, but differ at codon 118 of the ERCC1 gene. *Int J Oncol* 2000;**16**(3):555–60.
28. Plasencia C, Taron M, Martinez-Balibrea E, Rosell R, Abad A. Down regulation of thymidylate synthase gene expression after oxaliplatin administration: implication for the synergistic activity of sequential Oxaliplatin/5FU in sensitive and 5FU-resistant cell lines. American Association for Cancer Research (AACR) (New Orleans, LA). In: AACR Proceedings, vol. 42; 2001. p. 2735.
29. Carlini LE, Meropol NJ, Bever J, et al. UGT1A7 and UGT1A9 polymorphisms predict response and toxicity in colorectal cancer patients treated with capecitabine/irinotecan. *Clin Cancer Res* 2005;**11**(3):1226–36.
30. Tsuji T, Hidaka S, Sawai T, et al. Polymorphism in the thymidylate synthase promoter enhancer region is not an efficacious marker for tumour sensitivity to 5-fluorouracil-based oral adjuvant chemotherapy in colorectal cancer. *Clin Cancer Res* 2003;**9**(10 Pt 1):3700–4.
31. Meropol NJ, Gold PJ, Diasio RB, et al. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol* 2006;**24**(25):4069–77.
32. Metzger R, Danenberg K, Leichman CG, et al. High basal level gene expression of thymidine phosphorylase (platelet-derived endothelial cell growth factor) in colorectal tumours is associated with nonresponse to 5-fluorouracil. *Clin Cancer Res* 1998;**4**(10):2371–6.
33. Cobo M, Isla D, Massuti B, et al. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. *J Clin Oncol* 2007;**25**(19):2747–54.