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Multifactorial pharmacogenetic analysis in colorectal cancer patients receiving 5-fluorouracil-based therapy together with cetuximab–irinotecan

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Previous pharmacogenetic studies have reported the potential predictive value of *thymidylate synthase* (*TYMS*) polymorphisms or *methylenetetrahydrofolate reductase* (*MTHFR*) polymorphisms for the efficacy of 5-fluorouracil-based therapy, even though they have not yet been fully validated. Also, functional polymorphisms of genes linked to the epidermal growth factor receptor pathway [*epidermal growth factor* (*EGF*) and *epidermal growth factor receptor* (*EGFR*)], as well as polymorphisms of genes encoding for Fcγ receptors [*Fc fragment of IgG receptor 2A* (*FCGR2A*) and 3A (*FCGR3A*)], which influence their affinity for the Fc fragment, have been reported to be linked to the pharmacodynamics of cetuximab in the clinical setting.

WHAT THIS STUDY ADDS

- This prospective study conducted on advanced colorectal cancer patients receiving first-line tegafur-uracil–irinotecan–cetuximab therapy suggests that a favourable genotype score, considering both the *TYMS* 3RG allele and any Val-containing *FCGR3* allele, may be an indicator of better clinical response and longer overall survival.

AIM

To examine the predictive value of gene polymorphisms potentially linked to toxicity, clinical response, time to progression and overall survival, following cetuximab–tegafur-uracil (UFT)–irinotecan therapy.

METHODS

Fifty-two patients with advanced colorectal cancer were enrolled in an ancillary pharmacogenetic study of the phase II CETUFTIRI trial. Treatment consisted of 21 day cycles of cetuximab (day 1–day 8–day 15, 250 mg m^{-2} $week^{-1}$ following a 400 mg m^{-2} initial dose) together with irinotecan (day 1, 250 mg m^{-2}) and UFT–folinic acid (days 1–14, 250 mg m^{-2} day^{-1} UFT, 90 mg day^{-1} folinic acid). Analysed gene polymorphisms (blood DNA) were as follows: *EGFR* (CA repeats in intron 1, –216G>T, –191C>A), *EGF* (61A>G), *FCGR2A* (131Arg>His), *FCGR3A* (158Phe>Val), *UDP-glycosyltransferase1-polypeptide A1* (TA repeats), *TYMS* (28 bp repeats, including the G>C mutation on the 3R allele, 6 bp deletion in 3′ UTR) and *MTHFR* (677C>T, 1298A>C).

RESULTS

Maximum toxicity grade was linked to *EGFR* –191C>A polymorphism, with 71.1% grade 3–4 toxicity in CC patients *vs.* 28.6% in other patients ($P = 0.010$). A tendency to a better response was observed in patients bearing the *TYMS* 3RG allele ($P = 0.029$) and those bearing the *FCGR3A* 158Val genotype ($P = 0.020$). The greater the score of favourable *TYMS* and *FCGR3A* genotypes, the better the response rate ($P = 0.009$) and the longer the overall survival ($P = 0.007$). In multivariate analysis, the score of favourable genotypes was a stronger survival predictor than the performance status.

CONCLUSIONS

Present data suggest the importance of *FCGR3A* 158Phe>Val and *TYMS* 5′ UTR polymorphisms in responsiveness and survival of patients receiving cetuximab–fluoropyrimidine-based therapy.

Introduction

Colorectal cancer (CRC) is the second highest cause of cancer death in Western countries. Tegafur-uracil (UFT) is an oral fluoropyrimidine approved in the treatment of advanced colorectal cancer in several Western countries. The combination of irinotecan with 5-fluorouracil (5FU) and folinic acid (FA) [1] or with UFT–FA [2] results in significant antitumoural activity in metastatic CRC patients. The anti-epidermal growth factor receptor (EGFR) monoclonal antibody (mAb) cetuximab has demonstrated clinical activity in metastatic CRC in combination with irinotecan or oxaliplatin [3, 4]. Cetuximab acts by means of the following two independent mechanisms: the inhibition of EGFR signal transduction; and the possible activation of antibody-dependent cell cytotoxicity (ADCC). The ADCC is mediated by the Fc fragment of IgG1 mAbs, such as cetuximab. This fragment links target cancer cells to the Fc receptors (FcγR2a, FcγR3A) carried by immune cells, causing the lysis of target cells.

In the present study, cetuximab was given with oral UFT–FA and irinotecan, as first-line treatment in patients with metastatic colorectal carcinoma. This ancillary pharmacogenetic study was conducted on 52 of the 60 patients included in the French multicentre phase II study, CETUFTIRI. Our purpose was to analyse the possible relationships between treatment efficacy, or toxicity, and germinal gene polymorphisms linked to the administered drugs. We analysed the main functional polymorphism of the *UDP-glycosyltransferase1-polypeptide A1* (*UGT1A1*) gene (UGT1A1*28 variant), which affects the glucuronidation capacity of SN38, the active metabolite of irinotecan [5], along with the *DPYD*2A variant because the *DPYD* gene encodes for dihydropyrimidine dehydrogenase, the key enzyme of the 5FU catabolic pathway, as well as the following other gene polymorphisms relevant for fluoropyrimidine pharmacodynamics: the *TYMS* gene, coding for thymidylate synthase (TS), the main 5FU pharmacological target; and the *MTHFR* gene, coding for the methylenetetrahydrofolate reductase enzyme, controlling the intracellular reduced folate concentration, which is an essential cofactor for enhancing TS inhibition mediated by 5FU. Numerous studies have reported the potential predictive value of *TYMS* polymorphisms [6] or *MTHFR* polymorphisms [7] for the efficacy of 5FU-based therapy, even though they are not yet fully validated [8]. In our study, we also analysed functional polymorphisms of genes linked to the EGFR pathway, namely *EGF* and *EGFR* genes, as well as polymorphisms of genes encoding for Fcγ receptors (*FCGR2A* and *FCGR3A* genes), which influence their affinity for the Fc fragment. In fact, previous studies have suggested that *EGFR* and/or *EGF* polymorphisms [9–11] as well as *FCGR2A* and *FCGR3A* gene polymorphisms [11, 12] may explain interpatient variability in the pharmacodynamics of cetuximab.

Materials and methods

Patients and treatment

Patient recruitment was performed between December 2005 and December 2006, before *KRAS*-mutation testing was introduced as a requirement for cetuximab treatment. Inclusion criteria included patient age ≥18 years, histologically or cytologically confirmed, bidimensionally measured metastatic, unresectable CRC, Eastern Cooperative Oncology Group performance status 0 or 1, no prior chemotherapy, and adequate bone marrow and renal and hepatic function. The study was carried out with ethics committee approval. All patients received first-line therapy consisting of 21 day cycles of cetuximab (400 mg m^{-2} as initial dose, 250 mg m^{-2} for subsequent doses, i.v. over 2 h on days 1, 8 and 15), together with irinotecan (250 mg m^{-2} i.v. over 90 min on day 1) and UFT (250 mg m^{-2} day^{-1}) plus leucovorin (90 mg day^{-1}) daily from days 1 to 14. Treatment was administered until disease progression or unacceptable toxicity, for a maximum of eight cycles. A description of the 52 analysed patients is given in Table 1.

Toxicity evaluation

For each toxicity (leukopenia, neutropenia, thrombocytopenia, diarrhoea, nausea, vomiting, mucositis, asthenia,

Table 1

Patient characteristics ($n = 52$)

Age (years)	Mean	63.3
	Range	36–84
Gender	Men	33
	Women	19
Performance status (ECOG)	0	33
	1	19
Surgery on primary	No	13
	Yes	36
Adjuvant chemo- and/or radiotherapy	No	43
	Yes	9
Primary localization	Colon	34
	Rectosigmoid	7
	Rectum	11
Metastasis site	Liver	20
	Lung	1
	Peritoneum	1
	Lymph node	1
	Multiple sites	29
Number of cycles	Mean	5.8
	Median	7
	Range	1–8
Cumulative cetuximab dose (g m^{-2})	Mean	4.0
	Median	4.7
	Range	0.4–6.2
Cumulative UFT dose (g m^{-2})	Mean	19.3
	Median	22.4
	Range	0–28.0
Cumulative irinotecan dose (g m^{-2})	Mean	1.4
	Median	1.6
	Range	0–2.0

ECOG, Eastern Cooperative Oncology Group.

alopecia, acneiform rash, paronychia, hand–foot syndrome, anaphylactic shock, septic shock), the maximum observed toxicity grade was recorded (NCI-Common Terminology Criteria for Adverse Events v3.0). Then, for each patient, we considered the maximum observed toxicity grade (whatever the toxic pattern). In addition, we focused on the following factors: (i) the maximum observed neutropenia grade as a relevant indicator of irinotecan toxicity; and (ii) the score corresponding to the sum of rash and paronychia grades (score 0, 1 or 2 *vs.* score 3, 4, 5 or 6) as a relevant indicator of cetuximab-related cutaneous toxicity.

Efficacy evaluation

Best clinical response was assessed according to modified Response Evaluation Criteria in Solid Tumors [complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD)]. Time to progression (TTP) and survival were computed from day 1 of treatment. At the time of analysis, 51 patients of the 52 had progressed and 41 had died. As all 41 recorded deaths were cancer related, overall survival corresponded to specific survival. Median follow-up was 32.4 months (reverse Kaplan–Meier method).

KRAS *mutation analysis*

KRAS mutation analysis was performed retrospectively. Formalin-fixed, paraffin-embedded tumour material was collected from different pathology laboratories. In total, tumour material from 38 patients was collected. The percentage of tumour cells in analysed samples was ≥30%. DNA extraction (RecoverAll™ Kit from AMBION, Applied Biosystems, Courtaboeuf, France) and mutation analysis were centralized at the Centre Antoine Lacassagne, Nice. *KRAS* mutations at codon 12 and codon 13 were analysed according to a single-base extension multiplex assay adapted from Di Fiore *et al*. [13], on a Beckman CEQ 8000 sequencer. The following *KRAS*-characterized cell lines were used as controls: CCRF-CEM (mutated G12D), HCT116 (mutated G13D) and WiDr (wild-type). Nineteen patients of the 38 exhibited a *KRAS* mutation.

Pharmacogenetic analyses

On completion of patient recruitment, frozen blood samples (9 ml) were sent to the Centre Antoine Lacassagne (Nice), where DNA extractions were performed (Paxgene Blood DNA kit; QIAGEN, Courtaboeuf, France). Germinal polymorphisms of *TYMS*, *MTHFR*, *DPYD*, *EGFR*, *EGF*, *FCGR2A* and *FCGR3A* genes were analysed at the Centre Antoine Lacassagne, and *UGT1A1* polymorphism was analysed at the Centre Claudius Regaud (Toulouse).

The 28 bp repeat polymorphisms (2R or 3R, rs34743033) in the promoter region of the *TYMS* gene, along with the G>C mutation in the second repeat of the 3R allele (rs11540151), were analysed by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as previously described [14]. *TYMS* genotype was classified as a function of the number of theoretical E-box binding sites likely to bind Upstream Stimulatory Factor proteins, as follows: class 2 (2R2R or 2R3RC or 3RC3RC), class 3 (2R3RG or 3RC3RG) or class 4 (3RG3RG). The 6 bp deletion at position 1494 of the *TYMS* gene (rs11280056) was analysed by PCR and electrophoresis [15]. Polymorphisms at positions 677C>T (rs1801133) and 1298A>C (rs1801131) of the *MTHFR* gene were analysed according to melting curve analysis on LightCycler (Roche, Meylan, France) as previously described [15]. The *DPYD* IVS14+1G>A mutation (*DPYD**2A variant, rs3918290) was analysed with PCR-RFLP using the *Nde*I restriction enzyme [14]. The *EGFR* −216G>T (rs712829) and −191C>A (rs712830) polymorphisms were analysed by PCR-RFLP [16]. The CA repeats polymorphism in intron 1 of the *EGFR* gene (rs11568315) was investigated by means of fragment length analysis [17]. Owing to the large number of genotypes (between 15 and 22 CA repeats), patients were split into the following three groups: patients with both alleles <17 *vs.* patients with both alleles ≥17 *vs.* others. The *EGF* 61A>G (rs4444903), *FCGR2A* 131Arg>His (rs1801274) and *FCGR3A* 158Phe>Val (rs396991) gene polymorphisms were analysed by validated PCR-RFLP methods [18, 19]. The TA tandem repeat in the *UGT1A1* gene promoter was analysed by PCR using 5′-GCCAGTTCAACTGTTGTTGCC-3′ as forward primer and 5′-CCACTGGGATCAACAGTATCT-3′ as reverse primer. The UGT1A1*28 variant (rs8175347) corresponds to the [A(TA)$_7$TAA] sequence, while UGT1A1*1 (wild-type allele) corresponds to the [A(TA)$_6$TAA] sequence. The expected fragments (320 bp) were subjected to direct sequencing analysis with the Big dye terminator v3.1 cycle kit (Applied Biosystems, Warrington, UK). For UGT1A1 genotype, DNA samples from three patients with known TA$_6$/TA$_6$, TA$_6$/TA$_7$ and TA$_7$/TA$_7$ were used as controls. For other genotypes, wild-type and mutated cell lines were used as controls.

Statistics

The exact *P* values for Hardy–Weinberg equilibrium were tested on http://innateimmunity.net/IIPGA2. The nonparametric Kruskal–Wallis test was used to examine the influence of *UGT1A1* genotype on irinotecan dose. Fisher's exact test was applied to test the links between analysed genotypes and clinical end-points (CR + PR *vs.* SD + PD; toxicity grade or score ≤2 *vs.* toxicity grade or score >2), or between responsiveness and acneiform rash or *KRAS* status, or between toxicity and patient's characteristics. A logistic model was applied to estimate the odds ratio associated with toxicity markers (1 = grade or score >2, 0 = grade or score ≤2), response markers (1 = CR + PR, 0 = SD + PD) and for multivariate analysis. The TTP and survival curves were plotted according to the Kaplan–Meier method. The influence of the various tested parameters on TTP and survival was assessed by means of log rank test or Cox analysis (for continuous variables or multivariate analysis). Owing to the large number of tests performed, a *P* value of less than or equal to 0.010 was considered

statistically significant (two-sided tests). Statistics were performed using SPSS software (v15.0; SPSS Inc., Chicago, IL, USA).

Results

Description of toxicity and efficacy

The most frequent major toxicities were diarrhoea (2 grade 1, 10 grade 2, 8 grade 3) and acneiform rash (3 grade 1, 11 grade 2, 6 grade 3), followed by neutropenia (1 grade 1, 3 grade 2, 7 grade 3, 5 grade 4) and leukopenia (1 grade 1, 3 grade 2, 3 grade 3, 2 grade 4). Considering all toxicities, the highest toxicity recorded was grade 1 in 3 patients, grade 2 in 18 patients, grade 3 in 25 patients and grade 4 in 6 patients. Grade 3–4 toxicity was thus recorded in 59.6% of patients (31 of 52). Two patients developed anaphylactic shock at the first treatment cycle. Toxicity was not influenced by gender, age or Performance Status (PS).

Best clinical response, assessable in 49 patients, showed 3 CR, 21 PR, 11 SD and 14 PD, accounting for an overall response rate of 49%. Best response was significantly linked to the occurrence of an acneiform rash, with 65.5% response in patients developing grade 2–3 rash *vs.* 25% in those who did not [$P = 0.007$, odds ratio 5.7, 95% confidence interval (CI) 1.6–20.3]. Clinical response was higher in wild-type *KRAS* tumours compared with mutated *KRAS* tumours, even though the difference was not significant (64.7 *vs.* 47.4%, respectively, $P = 0.29$, odds ratio 2.0, 95% CI 0.53–7.8).

Median TTP was 5.7 months (95% CI 4.5–7.0). The TTP was not influenced by any demographic characteristics or treatment exposure (number of cycles, cumulative doses). Median overall survival was 18.6 months (95% CI 12.9–24.3). Overall survival was not related to demographic or therapeutic data other than PS (medians 20.9 *vs.* 9.9 months in patients with PS 0 and 1, respectively, $P = 0.027$), the number of administered cycles (median 18 months in patients with fewer than six cycles *vs.* 34 months in others, $P < 0.001$) and, as a corollary, the cumulative dose of cetuximab ($P < 0.001$), UFT ($P < 0.001$) and irinotecan ($P < 0.001$). The TTP and overall survival were not linked to *KRAS* mutation status.

Pharmacogenetic–pharmacodynamic relationships

Table 2 depicts the frequency of analysed genotypes, which were all in Hardy–Weinberg equilibrium. Of note, the irinotecan cumulative dose was not related to the *UGT1A1* gene polymorphism ($P = 0.54$). One patient of the 52 exhibited the IVS14+1G>A mutation (heterozygous) on the *DPYD* gene; this patient (36-year-old man) received two chemotherapy cycles at full UFT dose (treatment stopped for progression) and developed a maximum toxicity grade 3 mucositis, associated with a grade 2 acneiform rash and grade 1 diarrhoea, nausea, vomiting, asthenia, leukopenia and neutropenia.

Table 2

Distribution of gene polymorphisms

Gene	Genotype		*n*	%
TYMS	28 bp repeats	2R2R	10	19.6
		2R3R	29	56.9
		3R3R	12	23.5
	Class including G>C	2: 2R2R or 2R3RC or 3RC3RC	23	52.3
		3: 2R3RG or 3RC3RG	21	47.7
		4: 3RG3RG	0	0
	6 bp deletion	wt/wt	19	38.0
		wt/del	23	46.0
		del/del	8	16.0
MTHFR	677C>T	CC	20	40.0
		CT	27	54.0
		TT	3	6.0
	1298A>C	AA	24	48.0
		AC	24	48.0
		CC	2	4.0
UGT1A1	TA repeats	6/6	19	36.5
		6/7	26	50.0
		7/7	7	13.5
EGFR	CA repeats (intron 1)	Both alleles <17	13	25.0
		One allele <17, one allele ≥17	22	42.3
		Both alleles ≥17	17	32.7
	–216G>T	GG	17	33.4
		GT	27	52.9
		TT	7	13.7
	–191C>A	CC	38	73.1
		CA	14	26.9
		AA	0	0
EGF	61A>G	AA	19	36.5
		AG	27	52.0
		GG	6	11.5
FCGR2A	131Arg>His	Arg/Arg	13	25.0
		Arg/His	24	46.2
		His/His	15	28.8
FCGR3A	158Phe>Val	Phe/Phe	20	39.2
		Phe/Val	25	49.0
		Val/Val	6	11.8

The analysis of maximum toxicity grade, whatever the toxicity, revealed a marked tendency ($P = 0.010$) for patients bearing the *EGFR* –191C allele to develop greater toxicity, with 71.1% grade 3–4 toxicity in CC patients *vs.* 28.6% in CA patients (there was no AA patient), with an odds ratio of 6.13 (95% CI 1.58–23.79). In addition, an analysis focused on neutropenia demonstrated a strong tendency for deficient UGT1A1*28 patients to develop grade 3–4 neutropenia ($P = 0.011$); in comparison with *1/*1 patients, odds ratio was 3.13 (95% CI 0.57–17.2) for *1/*28 patients ($n = 26$) and 21.3 (95% CI 2.36–191.6) for *28/*28 patients ($n = 7$). Finally, analysis of cetuximab-related cutaneous toxicity, considering both acneiform rash and paronychia, revealed no influence of any *EGFR* or *EGF* polymorphism.

As regards efficacy, a trend was observed towards a better response in patients bearing the *TYMS* 3RG allele, i.e. belonging to the *TYMS* class 3 rather than class 2 (no class 4 in the present cohort, 65.0% response in class 3 *vs.* 28.6% in class 2, $P = 0.029$, odds ratio 4.64, 95% CI 1.24–17.37) and

Table 3

Impact of the favourable genotype score* on patient outcome

Favourable genotype score	Clinical response (complete response + partial response) Number of responsive patients/total number of patients	% Response	Odds ratio (95% confidence interval)	Overall survival Number of deaths/total number of patients	Median survival (months)	Relative risk of death (95% confidence interval)
0	1/11	9.1%	1	11/11	12.4	1
1	8/16	50.0%	10.0 (1.03–97.5)	15/19	14.7	0.49 (0.22–1.09)
2	9/13	69.2%	22.5 (2.11–240)	8/13	28.7	0.23 (0.09–0.60)
Overall statistics		Fischer's exact test $P = 0.009$			Log rank $P = 0.007$	

*The score corresponds to the number of favourable genotypes. Favourable genotypes are the class 3 *TYMS* genotype and any 158Val-containing *FCGR3A* genotype.

in patients bearing the *FCGR3A* 158Val genotype (62.1% response in Phe/Val or Val/Val *vs.* 26.3% in Phe/Phe, $P = 0.020$, odds ratio 4.58, 95% CI 1.29–16.27). We thus defined a favourable genotype score, considering both the class 3 *TYMS* genotype and any Val-containing *FCGR3* genotype (Table 3). The greater the favourable genotype score, the better the response rate, with 9.1, 50.0 and 69.2% response in patients with a score of 0, 1 and 2, respectively ($P = 0.009$; Table 3). In a bivariate analysis including the *KRAS* mutation status ($n = 29$ patients), the favourable genotype score was no longer significant.

The TTP was not influenced by any of the analysed gene polymorphisms, including the previously defined favourable genotype score.

In line with pharmacogenetic relationships reported on responsiveness, a longer, although nonsignificant, overall survival was observed in patients belonging to the *TYMS* class 3 genotype (median 26.4 months in class 3 *vs.* 14.4 months in class 2, $P = 0.041$; Figure 1) and in patients bearing the *FCGR3A* 158Val genotype (20.9 months in Phe/Val or Val/Val *vs.* 12.4 months in Phe/Phe, $P = 0.032$; Figure 2). As illustrated in Figure 3, the score of favourable *TYMS* and *FCGR3A* genotypes significantly influenced overall survival, with a median of 12.4 months in patients with no favourable genotype, 14.7 months in patients with one favourable genotype and 28.7 months in patients with two favourable genotypes (log rank, $P = 0.007$; Table 3). In addition, a bivariate Cox analysis including both the favourable genotype score and the PS showed that the genotype score ($P = 0.009$) was a stronger survival predictor than the PS ($P = 0.086$). Finally, adding *KRAS* mutation status in the multivariate model did not improve the above statistical significance (*P* values of 0.026, 0.083 and 0.61 for genotype score, PS and *KRAS*, respectively; $n = 30$ patients).

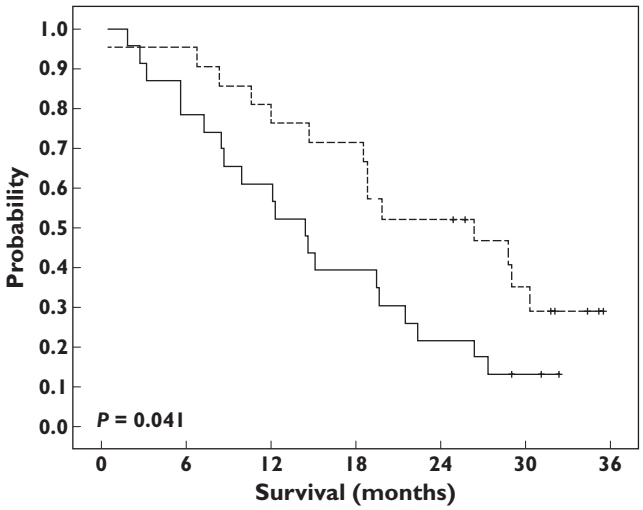

Figure 1

Overall survival probability according to *TYMS* genotype. Median overall survival was 14.4 months in class 2 patients (continuous line; 23 patients and 20 deaths) and 26.4 months in class 3 patients (dashed line; 21 patients and 14 deaths). Log rank test: $P = 0.041$. Class 2 (——); class 3 (– – –)

Discussion

The aim of this ancillary prospective study, conducted in 52 patients with metastatic CRC, was to perform a multifactorial pharmacogenetic analysis in patients receiving cetuximab in combination with irinotecan and tegafur-uracil plus folinic acid. To this end, we selected 11 relevant candidate gene polymorphisms that have previously been shown to influence the pharmacodynamics of cetuximab [9–12], irinotecan [5] or fluoropyrimidines [6, 7]. In contrast with the abundant literature on 5FU pharmacogenetics, little attention has been paid to the impact of *DPYD*, *TYMS* and *MTHFR* gene polymorphisms in the context of tegafur administration. However, such pharmacogenetic–pharmacodynamic relationships are supposed to be similar for 5FU and UFT, because p.o. UFT administration leads to 5FU systemic concentrations comparable to those observed after i.v. 5FU administration [20]. Pharmacogenetic studies on UFT have focused on functional *CYP2A6* polymorphisms, because CYP2A6 is responsible for the activation of tegafur into 5FU [21]; however, *CYP2A6*

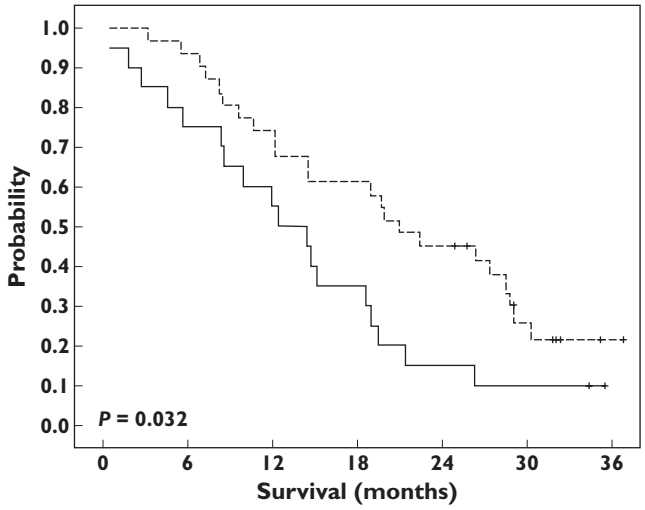

Figure 2

Overall survival probability according to *FCGR3A* 158Phe>Val genotype. Median overall survival was 12.4 months in Phe/Phe patients (continuous line; 20 patients and 18 deaths) and 20.9 months in Phe/Val or Val/Val patients (dashed line; 31 patients and 23 deaths). Log rank test: $P = 0.032$. Phe/Phe (——); Phe/Val or Val/Val (– – –)

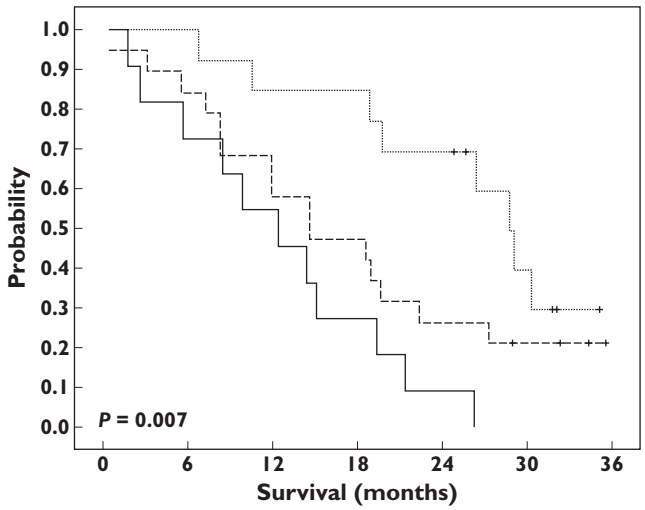

Figure 3

Overall survival probability according to the favourable genotype score, defined as class 3 *TYMS* genotype and Val-containing *FCGR3A* genotypes. Median overall survival was 12.4 months in patients with score 0 (continuous line; 11 patients and 11 deaths), 14.7 months in patients with score 1 (dashed line; 19 patients and 15 deaths) and 28.7 months in patients with score 2 (dotted line; 13 patients and 8 deaths). Log rank test: $P = 0.007$. Score 0 (——); score 1 (– – –); score 2 (·····)

polymorphisms have not been included in the present study because these variants are very rare in the Caucasian population and are more common among Asians [22].

An intrinsic difficulty of such analyses is the multifactorial nature of both toxicity and efficacy. Analysis of the global toxicity, whatever the toxicity pattern, shows no influence of either *TYMS* or *MTHFR* polymorphisms, in agreement with the study of Tsunoda *et al*. [23], conducted in 99 patients receiving UFT–FA, and that of Schwab *et al*. [24], conducted in 683 patients receiving 5FU, suggesting that these two genes play a limited role in fluoropyrimidine-related toxicity. However, these results constrast with those of Lecomte *et al*. [25] and Kristensen *et al*. [26], both reporting a significant relationship between 2R2R *TYMS* genotype and an increased risk of grade 3–4 toxicity, in 90 and 68 colorectal cancer patients receiving 5FU-based treatment, respectively. Also, a recent study from Afzal *et al*. [27] reported that 677C>T and 1298A>C *MTHFR* genotypes associated with the greatest enzyme expressions were predictive of gastrointestinal toxicity after 5FU-based treatment. In the present study, the analysis of global toxicity revealed a marked influence of *EGFR* –191C>A polymorphism, with CC patients being more exposed to grade 3–4 toxicity compared with CA patients (odds ration 6.13, $P = 0.01$). Regarding cetuximab-related cutaneous toxicity, however, none of the analysed *EGFR* polymorphisms (–216G>T, –191C>A, CA repeats in intron 1) showed a significant predictive value. The lack of prediction of intron 1 CA repeats on skin toxicity has recently been reported in cetuximab-treated patients [28] and contrasts with previous data from Amador *et al*. [29] and Graziano *et al*. [9], who demonstrated greater skin toxicity in anti-EGFR-treated patients exhibiting fewer CA repeats in intron 1 of the *EGFR* gene. We also examined the impact of *UGT1A1**28 polymorphism on neutropenia, because neutropenia is a limiting toxicity of irinotecan, although it may also be induced by UFT. The UGT1A1 enzyme governs the glucuronidation of SN38, the active metabolite of irinotecan, and numerous studies have shown that patients deficient for UGT1A1 enzyme, i.e. bearing the *UGT1A1**28 variant, were prone to a lower SN38 glucuronidation rate and developed more severe neutropenia [5, 8, 30, 31]. Accordingly, the present data show a strong tendency for homozygous- and heterozygous-deficient patients to be at risk for developing grade 3–4 neutropenia, compared with homozygous nondeficient patients (odds ratios 21.3 and 3.13, respectively).

Patient recruitment was done before *KRAS* analysis was required for initiating cetuximab therapy. In line with data in the literature [32], response rate was higher in patients with a wild-type *KRAS* tumour compared with patients having a mutated *KRAS* tumour, although this difference did not reach significance (odds ratio 2.0, 95% CI 0.5–7.8). Also, response rate was significantly higher in patients developing skin toxicity (odds ratio 5.7, 95% CI 1.6–20.3), in agreement with previous studies [33, 34]. A pharmacological explanation for such a relationship may lie in pharmacokinetic variability, as suggested by Fracasso *et al*. [35], who reported higher cetuximab serum concentrations in patients with partial response/stable disease compared with patients having progressive disease. As for skin toxic-

ity, we did not observe a significant link between *EGFR* gene polymorphisms and clinical response. This contrasts with data of Graziano *et al*. [9] reporting that patients with fewer CA repeats in intron 1 had both a higher response rate and skin toxicity, suggesting an additional pharmacogenetic explanation for the relationship between skin toxicity and cetuximab responsiveness.

In the present study, a trend was observed towards both a higher response rate and a longer overall survival in patients bearing the *TYMS* 3RG allele, as well as in patients bearing the *FCGR3A* 158Val allele. Interestingly, as illustrated in Table 3 and Figure 3, when combining *TYMS* 3RG and *FCGR3A* 158Val genotypes, the greater the number of favourable genotypes, the higher the response rate ($P = 0.009$) and the longer the overall survival ($P = 0.007$). Importantly, overall survival was significantly linked to PS, and a multivariate analysis including PS showed that the number of favourable genotypes was a significantly stronger survival predictor than PS.

Numerous studies have shown that elevated TS protein or mRNA expression is generally associated with poor outcome in patients receiving exclusive 5FU-based chemotherapy [36]. However, the impacts of *TYMS* gene polymorphisms on fluoropyrimidine pharmacodynamics are more conflicting [6], with some studies demonstrating a deleterious impact of *TYMS* 3RG genotypes [14, 37–39], whereas others do not [40, 41], and other investigators reporting a favourable impact of the 3R allele [42, 43]. In the majority of the above-mentioned studies [14, 37–42], as well as in the present one, *TYMS* genotyping was performed on blood DNA. Of note, *TYMS* gene is localized on the short arm of chromosome 18, which is prone to frequent deletions in colorectal cancers [44], thus resulting in loss of heterozygosity at the *TYMS* locus in the tumour [45]. As suggested in two clinical studies which analysed *TYMS* 2R3R genotype in both tumoural and blood DNA, *TYMS* gene polymorphism measured in blood DNA is not as relevant as *TYMS* gene polymorphism measured in tumour for predicting outcome of 5FU-treated patients [43, 46]. This observation may explain the inconsistency of *TYMS* pharmacogenetic–pharmacodynamic relationships in the literature.

Numerous *in vitro* studies have demonstrated that blood mononuclear cells, or NK cells, mediate cetuximab-induced ADCC against different cancer cell line types [47–50]. Furthermore, it has been shown that *in vitro* cell cytotoxicity was significantly higher with effector cells expressing *FCGR3A* 158Val/Val genotype compared with Phe/Val or Phe/Phe genotypes [48, 49]. This latter observation is in agreement with IgG binding experiments which demonstrated that anti-CD20 or anti-CD16 IgG1 mAbs display greater affinity for FcγR3A receptors carried by NK cells isolated from *FCGR3A* 158Val/Val individuals compared with *FCGR3A* 158Phe/Phe subjects [51, 52]. Taken together, these data suggest that *FCGR3A* 158Phe>Val genotype may influence the efficacy of cetuximab-based therapy, via ADCC. Accordingly, we report that patients carrying the *FCGR3A* 158Val allele exhibited a higher response rate and a longer survival than homozygous 158Phe/Phe patients, in line with data in the literature showing that the *FCGR3A* Val allele is associated with an improved outcome in patients treated with cetuximab [12] or with other IgG1 mAbs, such as rituximab [19, 53, 54] or trastuzumab [55]. This consistent finding that *FCGR3A* 158Val allele enhanced IgG1 mAb efficacy contrasts with a single study published by Pander *et al*. [11] showing the opposite pattern on 122 metastatic CRC patients receiving cetuximab together with bevacizumab, capecitabine and oxaliplatin, with longer progression-free survival in *FCGR3A* 158Phe/Phe patients ($P = 0.025$). Also, three studies did not reveal any significant relationship between *FCGR3A* 158Phe>Val genotype and outcome of metastatic CRC patients receiving cetuximab alone (35 and 127 patients, respectively) [10, 56] or in combination with irinotecan (110 patients) [9]. The therapeutic impact of ADCC *in vivo* is likely to be of secondary importance, as suggested by the lack of objective response observed in *KRAS*-mutated patients receiving cetuximab as monotherapy.

In conclusion, our present data suggest the importance of *FCGR3A* and *TYMS* gene polymorphisms in responsiveness and overall survival of patients receiving cetuximab–UFT based therapy. Engineering approaches of mAbs are currently being developed to enhance ADCC by increasing the affinity of mAb to Fcγ receptors. To this end, protein- and glyco-engineering of the mAb Fc region have recently been applied to cetuximab and have proved to be effective against *KRAS*-mutated tumours *in vitro* [57]. Such approaches open up promising prospects for improving anti-EGFR therapy in metastatic cancer patients and are presently being tested in clinical trials.

Competing Interests

EF has received fees for speaking and consulting from Merck Laboratories. There are no other competing interests to declare.

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