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Phase II study of pharmacogenetic-tailored therapy in elderly colorectal cancer patients☆

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ABSTRACT

Background: Retrospective analyses suggested that a pharmacogenetic approach may allow a tailored selection of chemotherapy for metastatic colorectal cancer.
Aim: We conducted a phase II study of pharmacogenetic-selected first-line chemotherapy in elderly patients with advanced colorectal cancer, with the aim to improve efficacy and to reduce toxicity in this group of patients.
Methods: 24 patients were enrolled in this study. Chemotherapy regimen was prospectively assigned based on TS, DPD, ERCC-1 and UGT1A1 genotyping results. Twelve patients (50%) were treated with modified FOLFIRI, 11 patients (46%) with modified FOLFOX6 and 1 (4%) with De Gramont regimen.
Results: A partial remission was obtained in 4 cases (17%), stable disease in 8 cases (33%) and progressive disease in 12 cases (50%). Grade 3–4 neutropenia was observed in 7 patients (29%) and diarrhoea in 3 cases (12%). The trial was then interrupted according to study design requiring 13 partial remissions out of the first 24 patients enrolled as the necessary response rate level in order to continue.
Conclusion: Prospective selection of chemotherapy based on TS, DPD, ERCC-1 and UGT1A1 expression in elderly advanced colorectal cancer patients failed to confirm previous results. A more accurate validation of retrospective findings is warranted before these molecular markers can be used for treatment selection in the clinical practice.

1. Introduction

The proportion of the population over the age of 70 years is expected to increase from a quarter to one third over the next two decades in Western Countries and the prevalence of elderly patients with colorectal cancer is then expected to increase accordingly [1–4].

In the metastatic setting the use of chemotherapy and targeted agents has improved survival, control of symptoms and quality of life. Unfortunately in presence of elderly patients a progressive decrease in the use of standard chemotherapy has been also observed [3]. However our knowledge about the real impact of advanced age on both tumour biology and treatment outcome is limited mainly because of the small proportion of elderly patients included in clinical trials. The lack of research data along with the physiologic declining in organ function and the presence of co-morbidities often translates into an actual difficulty in delineating an effective treatment strategy for aged patients with metastatic colorectal cancer. A growing body of pre-clinical and retrospective findings seems to indicate that genomic polymorphisms in genes implied in drug metabolism and activity could have crucial implications for chemotherapy efficacy and toxicity. It has been suggested that a pharmacogenetic approach may then represent a crucial strategy for optimizing treatment selection [5–7].

Over-expression of the 5-Fluorouracil (5-FU) target enzyme thymidilate synthase (TS) has been correlated with a poorer outcome in colorectal cancer patients treated with 5-FU. Moreover the variable number of tandem repeat (VNTR) polymorphism in TS 5′-untranslated region (5′-UTR), consisting of 2 (2R) or 3 (3R) 28-bp repeated sequences as well as a G/C polymorphism in the 3R allele determining 2 additional alleles at this locus (3RG or 3RC) have been shown to determine altered levels of expression of TS

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Table 1
Description of genotypes analysed and primer sequences.

Gene and site	Polymorphism	Genotype		Primer Sequence
TS (5′-UTR)	VNTR and SNP	2R/3R alleles CIG SNP in 3R carriers	F	GTGGCTCCTGCGTTTCCCCC
			R	CTCCGAGCCGGCCACAGG
TS (3′-UTR)	SNP	6+/6−	F	CAAATCTGAGGGAGCTGAGT
			R	CAGATAAGTGGCAGTACAGA
ERCC1 (exon 4)	SNP	C/T Rs11615	F	GAGAGGGCTGAGCTGGAGACAG
			R	CCAGCACATAGTCGGGAATTACGTC
DPD (exon/intron 14 splice site)	SNP	IVS14 + 1G > A Rs3918290	F	GAACCACCTCTGGCCCCACGTATG
			R	GCATCAGCAAAGCAACTGGCAG
UGT1A1*28 (promoter)	VNTR	$(TA)_6$ $(TA)_7$	F	CAGCCTCAAGACCCCACA
			R	TGCTCCTGCCAGAGGTTC

TS, thymidilate synthase; UTR, untranslated region; ERCC1, excision repair cross complementing group 1; DPD, diihydropyrimidine dehydrogenase; UGT1A1*28, uridine diphosphate glucuronosyltransferase 1A1*28; VNTR, variable number of tandem repeat; SNP, single nucleotide polymorphism; F, forward; R, reverse.

and consequently to affect clinical outcome during 5-Fluorouracil chemotherapy [8–12]. Similar considerations may be applicable to a polymorphism (a 6-bp insertion/deletion) in the TS 3′UTR that has been reported to influence TS gene expression with higher TS mRNA levels in 6+/6+ carriers [5,6,13].

Similarly resistance to platinum derivatives such as oxaliplatin has been attributed to enhanced tolerance and repair of DNA damage through proteins of the nucleotide excision repair (NER) pathway, which includes the excision repair cross complementing group 1 (ERCC1) [5–7,14]. Low expression of ERCC1 as in presence of the ERCC1-118C allele variant seems to correlate with improved response during treatment with platinum compounds. On the contrary the ERCC1-118T allele variant has been correlated with a trend for a higher ERCC1 mRNA levels and a consequently adverse effect on patients outcome.

Dihydropyrimidine dehydrogenase (DPD) is the rate limiting enzyme for 5-FU catabolism. As a consequence of DPD deficiency patients treated with 5-FU are exposed to an increased levels of active metabolites and to an increased toxicity [15].

Analogously the uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) is a key enzyme in the metabolism of irinotecan. The UGTA1A1*28 polymorphism characterised by an extra TA repeat in the promoter region of the gene is believed to be involved in irinotecan toxicity. The $(TA)_7$ variant is associated with reduced glucuronidation activity of the enzyme, increased irinotecan metabolites accumulation and hence increased toxicity [16–18].

Taken together these findings may be of particular interest in an elderly patients population, in whom the need for maximizing efficacy and reducing toxicity is clearly crucial.

On these bases we conducted a phase II prospective trial in an elderly population with advanced colorectal cancer. The first-line chemotherapy regimen was assigned after and basing on genotyping results of TS (TS 5′-UTR, TS 3′-UTR), ERCC1, DPD and UGT1A1*28. Primary end-points were improved response rate and reduced toxicity.

2. Materials and methods

2.1. Patients selection

Eligibility criteria included: histologically proven metastatic colorectal adenocarcinoma, patient's age ≥70 years, an ECOG performance status 0–1; adequate haematological, hepatic, renal and cardiac function. Exclusion criteria included: previous malignancies other than superficial skin cancer or in situ cervical carcinoma. The trial was approved by local ethics committees. All patients provided informed consent prior to study inclusion.

2.2. Genotyping

Genotyping of TS, ERCC1, DPD and UGT1A1 was performed from patients' genomic DNA.

Genomic DNA was extracted from 2 ml of whole blood by FlexiGene DNA kit (Qiagen Inc., Valencia, CA, United States), following the manufacturer's instructions. Concentration and purity index of each sample were evaluated by UV spectrophotometry as the ratio absorbance 260/280 nm; a purity index of 1.5–2.0 was considered optimal.

The polymorphic site in the 3′UTR region of TS gene, the SNPs-containing fragments of ERCC1 and DPD genes, along with the (TA) repeats tract in the promoter of UGT1A1 were PCR-amplified in a 50 μl reaction solution, containing 200 ng of DNA, 10 μl of 10 mM dNTPs (Sigma–Aldrich, St. Louis, MO, United States), 2.5 U of platinum Taq DNA polymerase, 2 mM magnesium chloride, 5 μl of magnesium-free 10× PCR buffer (Invitrogen Corp., Carlsbad, CA, United States) and 50 pmol of each primer (primer sequences are shown in Table 1). The promoter region of the TS gene containing the 28-bp polymorphic repeat, was amplified at similar conditions, except for magnesium chloride (1.5 mM) and for the addition of 10 μl of 100% glycerol. PCR conditions were: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 30 s at the primer-specific annealing temperature, as reported in Table 1, and 72 °C for 40 s, with a final extension step at 72 °C for 7 min on a MyCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, United States). PCR products were quality tested by a 3% agarose gel analysis and then purified by PCR Purification kit (Qiagen), following the manufacturer's instructions.

The sequence variants of TS, ERCC1 and DPD were analysed by RFLP, according to standard protocols, as previously described. Briefly, 10–15 μl of each amplification product were digested using 20 U of the appropriate restriction enzyme: HaeIII for TS 5′UTR, DraI for TS 3′UTR and HpyCH4IV for DPD and ERCC1 (New England Biolabs Inc., Beverly, MA, United States). After an incubation for 90 min at 37 °C, the tested variants were characterised by the digestion products electrophoretic pattern shown in a 4% agarose gel run analysis.

UGT1A1*28 was distinguished from the most common allele (UGT1A1*1) by direct sequencing on an ABI PRISM 310 Genetic Analyzer, using the ABI Prism DNA Sequencing kit (Applied Biosystems, Foster City, CA, United States). All samples were independently genotyped in duplicate.

2.3. Treatment plan

Selection of the first-line chemotherapy regimen was made based on genotyping results as shown in Tables 2 and 3.