# DPD and UGT1A1 deficiency in colorectal cancer patients receiving triplet chemotherapy with fluoropyrimidines, oxaliplatin and irinotecan

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## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Pharmacogenetic testing of *DPYD* and *UGT1A1* is recommended in clinical practice prior to administration of fluoropyrimidines and irinotecan, respectively.
- Only uncommon variants of *DPYD* are recommended, since more frequent functional variants (c.496A > G, c.1129-5923C > G and c.1896 T > C) are controversially associated with severe toxicity.

## AIMS

Triplet chemotherapy with fluoropyrimidines, oxaliplatin and irinotecan is a standard therapy for metastatic colorectal cancer (CRC). Single nucleotide polymorphisms (SNPs) in *DPYD* and *UGT1A1* influence fluoropyrimdines and irinotecan adverse events (AEs). Low frequency *DPYD* variants (c.1905 + 1G > A, c.1679 T > G, c.2846A > T) are validated but more frequent ones (c.496A > G, c.1129-5923C > G and c.1896 T > C) are not. rs895819 T > C polymorphism in hsa-mir-27a is associated with reduced DPD activity. In this study, we evaluated the clinical usefulness of a pharmacogenetic panel for patients receiving triplet combinations.

## METHODS

Germline DNA was available from 64 CRC patients enrolled between 2008 and 2013 in two phase II trials of capecitabine, oxaliplatin and irinotecan plus bevacizumab or cetuximab. SNPs were determined by Real-Time PCR. We evaluated the functional variants in *DPYD* (rare: c.1905 + 1G > A, c.1679 T > G, c.2846A > T; most common: c.496A > G, c.1129-5923C > G, c.1896 T > C), hsa-mir-27a (rs895819) and *UGT1A1* (*28) genes to assess their association with grade 3–4 AEs.

## RESULTS

None of the patients carried rare *DPYD* variants. We found *DPYD* c.496A > G, c.1129-5923C > G, c.1896 T > C in heterozygosity in 19%, 5% and 8%,

### WHAT THIS STUDY ADDS

- We showed that more frequent functional variants of *DPYD* and concomitant *UGT1A1* assessment are relevant in patients receiving both fluoropyrimidines and irinotecan as part of an intensive triplet chemotherapy strategy.
- The role of hsa-mir-27a in epigenetic regulation of *DPYD* was explored for the first time in this study in patients receiving intensive triplet chemotherapy with fluoropyrimidines, oxaliplatin and irinotecan.

respectively, homozygous rs895819 in hsa-mir-27a in 9% and homozygous *UGT1A1*28* in 8%. Grade 3–4 AEs were observed in 36% patients and were associated with *DPYD* c.496A > G (odds ratio (OR) 4.93, 95% CI 1.29, 18.87; $P = 0.021$) and homozygous rs895819 in hsa-mir-27a (OR 11.11, 95% CI 1.21, 102.09; $P = 0.020$). Carriers of *DPYD* c.1896 T > C and homozygous *UGT1A1*28* showed an OR of 8.42 (95% CI 0.88, 80.56; $P = 0.052$). Multivariate analysis confirmed an independent value for *DPYD* c.496A > G and c.1896 T > C.

### CONCLUSIONS
Concomitant assessment of *DPYD* variants and the *UGT1A1*28* allele is a promising strategy needing further validation for dose personalization.

## Introduction

From the era of 5-fluorouracil (5-FU), substantial improvements in survival of patients with advanced colorectal cancer (CRC) were achieved via the introduction of newer chemotherapeutic agents (oxaliplatin and irinotecan) and biological agents targeting angiogenesis (such as bevacizumab) and the epidermal growth factor receptor (such as cetuximab and panitumumab). It is well known that exposure to all three active chemotherapy agents in the course of the disease is associated with a survival benefit, irrespective of their sequence [1, 2]. In two Italian phase III studies, triplet chemotherapy with 5-FU, oxaliplatin and irinotecan (FOLFOXIRI regimen) was superior to doublet chemotherapy in terms of efficacy and also in the setting of bevacizumab-based treatment [3, 4]. Toxicity was significantly increased in patients receiving FOLFOXIRI, particularly in terms of diarrhoea, neutropenia, mucositis and asthenia. Initially, this concern limited the widespread use of this regimen in the real-world clinical practice setting outside of well selected patients or clinical trials [3, 4].

Genetic variations in genes involved in drug metabolism are partially responsible for the inter-patient variability in the occurrence of toxicity. Regarding fluoropyrimidine-associated toxicity, single nucleotide polymorphisms (SNPs) in the dihydropyrimidine dehydrogenase (DPD) gene (*DPYD*) have been recognized as predictive risk alleles. The genotyping of c.1905 + 1G > A, c.1679 T > G and c.2846A > T is used in clinical practice to prevent severe toxicity The Clinical Pharmacogenetics Implementation Consortium Guidelines recommend an alternative drug for *DPYD*2A*, *13* or rs67376798 homozygotes and a starting dose of 50% (or less) for the heterozygotes followed by titration of dose according to drug tolerability and toxicity [5]. However, such SNPs explain only a small percentage of the toxicity due to low allelic frequency, since their minor allele frequency (MAF) in the general population is estimated to be <0.01 (1000 Genomes Project Phase 1 allele frequencies). More frequent *DPYD* variants such as *DPYD* c.1896 T > C mapping closely to *DPYD* c.1905 + 1G > A [6], the deep intronic variant c.1129-5923C > G affecting the DPD pre-mRNA splicing [7] and the *DYPD* c.496A > G [8] have been associated with the occurrence of severe toxicities with conflicting results. Additionally, recent papers reported that inter-individual differences in *DPYD* expression can arise also from epigenetic factors. For instance, miR-27a and miR-27b may repress *DPYD* expression [9, 10] and variant alleles of rs895819 mapping in the coding region of the hsa-mir-27a were associated with reduced DPD enzyme activity [10]. Regarding irinotecan, UDP-glucuronosyltransferase (UGT1A1) plays an important role in the metabolism of its active metabolite, i.e. SN-38. The *UGT1A1*28* allele affects gene expression and leading to decreased glucuronidation of the metabolite SN-38 and increased risk of severe irinotecan-induced neutropenia [11]. The *UGT1A1*28* genotype can be used to individualize the dosing of irinotecan [12].

The aim of our study was to elucidate the clinical relevance of a pharmacogenetic model including the major functional variants that may affect the safety outcome of the patients receiving triplet chemotherapy for advanced CRC.

## Methods

### *Patients population and treatment*
This was a prospective, observational, monocentric study approved by the Local Ethics Committee of the Fondazione IRCCS Istituto Nazionale dei Tumori (National

Cancer Institute of Italy). From 2008 to 2013, 64 patients with advanced CRC were enrolled at our Institution in two phase II trials of capecitabine, oxaliplatin and irinotecan (COI regimen) plus a monoclonal antibody, i.e. bevacizumab [multicentre protocol COI-B; EudraCT No. 2008-008749-39] or cetuximab [monocentre protocol COI-E; EudraCT No. 2008-001062-93]. All patients provided written informed consent prior to any procedure of this optional ancillary sub-study. Inclusion criteria were those of the main clinical studies including histologically confirmed, advanced CRC; age >18 years, life expectancy ≥12 weeks, ECOG performance status ≤1 and adequate renal, hepatic and bone marrow function, in particular total bilirubin level within laboratory range and alkaline phosphatase level lower than 2.5 times the upper normal level. To unmask the effect of the variants in study, an additional inclusion criterion was the absence of c.1905 + 1G > A, c.1679 T > G and c.2846A > T *DPYD* variants. Patients received intravenous 1 h infusion of irinotecan at the dose of 180 mg m$^{-2}$ and bevacizumab at the dose of 5 mg kg$^{-1}$ or cetuximab at the dose of 500 mg m$^{-2}$ on day 1, 3 h infusion oxaliplatin at the dose of 85 mg m$^{-2}$ on day 2 and 1000 mg m$^{-2}$ of oral capecitabine twice daily from days 2 to 6. Cycles were repeated every 2 weeks for a maximum of eight cycles or until progressive disease (PD), patient's refusal or inacceptable toxicity. In protocol COI-B, treatment was administered with a palliative purpose [13]. In protocol COI-E, patients received the treatment peri-operatively before and after resection of liver metastases with curative intent [14]. Demographic data, medical history and adverse drug reactions were collected. Assessment of chemotherapy-induced specific toxicity was based on the National Cancer Institute's Common Terminology Criteria (NCI-CTC v. 4.0) for Adverse Events (AEs). We considered the worst grade of toxicity for each patient. Explicit supportive care guidelines were in place in the event of diarrhoea. For severe episodes, 2 mg loperamide was administered every 2 h during the day and every 4 h at night, until resolution of symptoms. Acute diarrhoea was prevented by prophylactic atropine with standard protocol. Dose reductions of chemotherapy, as well as treatment delays and permanent discontinuations were also recorded.

## Genotyping

Genomic DNA was isolated from peripheral blood cells using an automatic DNA extraction system (Maxwell® 16 System, Promega) according to the manufacturer's instructions. DNA concentration and purity were evaluated by absorbance methodology using a NanoDrop 1000 Spectrophotometer V3.7 (Thermo Scientific). SNPs were determined by real-time PCR, using a panel of LightSNiP from TIB-MolBiol (assays based on SimpleProbe®). The SimpleProbe® included in the LightSNP assay can detect single base mismatches, thus enabling analysis of polymorphisms. At the end of the amplification a melting

curve analysis was performed (LightCycler 480, Roche). We evaluated the functional variants mapping in *DPYD* (low frequency: c.1905 + 1G > A/rs3918290, c.1679 T > G/rs55886062, c.2846A > T/rs67376798; high frequency: c.496A > G/rs2297595, c.1129-5923C > G/rs75017182, c.1896 T > C/rs17376848), hsa-mir-27a (rs895819) and *UGT1A1* (*28*/rs8175347) genes.

## Statistical analysis

The primary endpoint was the correlation between high frequency SNPs and severe toxicity. Secondary endpoints were their correlation with timing of toxicity and treatment protocol modification. The Hardy–Weinberg equilibrium was evaluated using the $\chi^2$ test [15]. AEs were dichotomized as either mild to moderate (grade 0–2) or severe (grade 3–4). Association between chemotherapy-associated grade 3–4 AEs and selected genotypes was assessed using the Fisher exact test. *P* values were compared with the significance threshold of 0.05. Polymorphisms associated with severe toxicity in univariate analysis with a *P* value inferior to 0.10, entered in a multivariate logistic regression model, to evaluate their overall effect on occurrence of severe AEs. Since the results of this study were considered hypothesis-generating, we did not perform corrections for multiple testing according to Streiner *et al*. [16]. Analyses were performed using SAS 9.4.

# Results

## Patient characteristics

Patient and treatment characteristics are shown in Table 1. Briefly, the present study enrolled 39 males and 25 females. ECOG Performance Status was classified as 0 in almost all patients (62 out of 64, 97%). All patients received at least one cycle of treatment and were evaluable

**Table 1**

Patients' characteristics

| Baseline characteristics | Total n (%) | COI-B n (%) | COI-E n (%) |
|---|---|---|---|
| **Total** | 64 (100) | 36 (100) | 28 (100) |
| Male | 39 (61) | 19 (53) | 20 (71) |
| Female | 25 (39) | 17 (47) | 8 (29) |
| **Median age, years (range)** | 57 (34–73) | 55 (34–72) | 58 (36–73) |
| **Patients with grade 3–4 adverse events** | 23 (36) | 8 (22) | 15 (53) |
| Diarrhoea events | 20 (31) | 7 (19) | 13 (46) |
| Neutropenia events | 3 (5) | 1 (3) | 2 (7) |
| Asthenia events | 2 (3) | 0 (0) | 2 (7) |
| **Treatment protocol modifications** | 29 (45) | 14 (39) | 15 (54) |
| Dose reduction or dose delay | 22 (34) | 12 (34) | 10 (36) |
| Permanent discontinuation | 7 (11) | 2 (5) | 5 (18) |

COI-B: capecitabine, oxaliplatin and irinotecan with bevacizumab; COI-E: capecitabine, oxaliplatin and irinotecan with cetuximab.

for toxicity. In particular, 36 (56%) patients received triplet chemotherapy with bevacizumab, while 28 (44%) received cetuximab. The median number of cycles was 8 (range 1–8). A total of 23 (36%) patients developed grade 3–4 chemotherapy-related AEs. In particular, among patients with severe AEs, 10 (43%) were treated with the COI-B and 13 (57%) with the COI-E regimen. Grade 3–4 toxicities were mainly represented by diarrhoea (31%), followed by a low incidence of neutropenia (5%) and asthenia (3%). Grade 3 neurotoxicity attributable to oxaliplatin was registered. No acute irinotecan-related episodes of diarrhoea were registered. No significant differences of severe toxicity according to age and gender were observed. Dose reductions or delays were necessary in 22 (34%) patients, after a median time of 6 weeks. Permanent treatment discontinuation was necessary in seven (11%) cases. There was a higher incidence of toxicity in the cetuximab (COI-E) vs. the bevacizumab group (COI-B), as shown in Table 1 ($P = 0.017$). However, the type of monoclonal antibody used was not associated with treatment protocol modifications ($P = 0.28$).

## Case analysis

All selected functional SNPs were successfully genotyped in our cases. Since no c.1905 + 1G > A, c.1679 T > G and c.2846A > T rare *DPYD* variants were detected, all screened patients were included in the study. *DPYD* variants c.496A > G, c.1129-5923C > G and c.1896 T > C were present only in heterozygosis in 12 (19%), three (5%) and five (8%) patients, respectively. Variant alleles

in the hairpin loop region of hsa-mir-27a rs895819 were present in heterozygosis in 27 (42%) patients and in homozygosis in six (9%), respectively. Thirty-two (50%) patients carried the *UGT1A1*28* allele in heterozygosis and five (8%) in homozygosis. All SNPs were found to be in Hardy–Weinberg equilibrium ($P > 0.05$). As shown in Figure 1, six patients showed the concomitant presence of more than one SNP. In particular, hsa-mir-27a rs895819 in homozygosis accompanied *DPYD* variant c.496A > G and *UGT1A1*28/*28* in four and one patients, respectively.

## Association between SNPs and toxicity

In the calculation of OR, we used a dominant genetic model for *DPYD* since all SNPs were found in heterozygosis. For *UGT1A1* and hsa-mir-27a, which were found in both heterozygosis and homozygosis, we used a dominant and recessive model. However, since only recessive models were associated with toxicity, herein we reported only this type of analysis. Table 2 lists the associations between functional SNPs and grade 3–4 chemotherapy-induced AEs. The *DPYD* c. 496 G risk allele was significantly associated with grade 3–4 AEs ($P = 0.021$), with an OR of 4.93 (95% CI 1.29, 18.87). No significant association of the *DPYD* c.1129-5923C > G variant with severe toxicity was detected ($P = 1$). The risk analysis demonstrated a non-significant trend for association when considering the heterozygosity status of the *DPYD* c.1896 T > C variant (OR = 8.42; 95% CI, 0.88, 80.56; $P = 0.052$). Carriers of variant C allele in homozygous status at
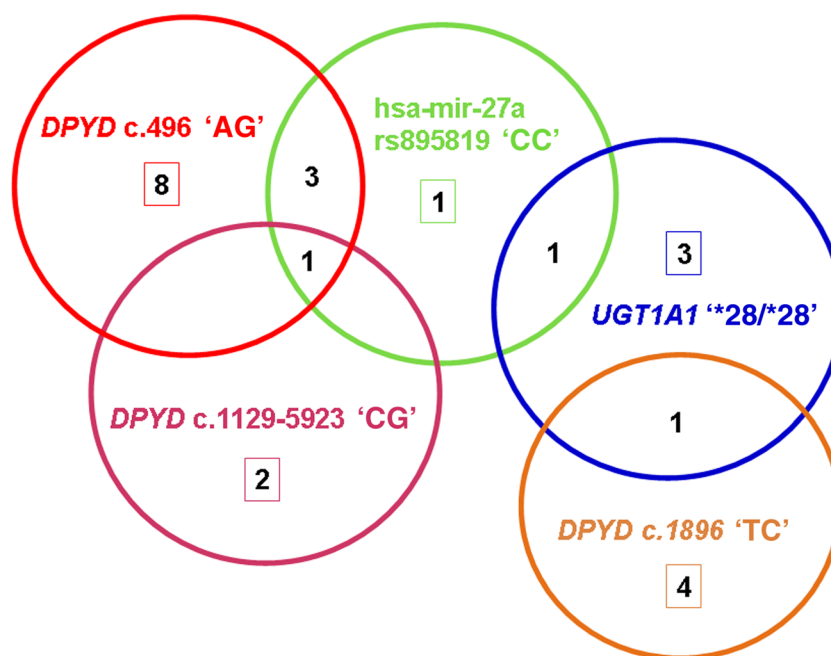


**Figure 1**

Individual frequencies of the selected polymorphisms in our series. The numbers indicate the number of patients carrying the 'risk genotype'

## Table 2

Genetic analysis of SNPs in *DPYD*, hsa-mir-27a, *UGT1A1* and chemotherapy-induced AEs

| Gene | Variant | MAF* (%) | Effect | Genotype | Genetic model | Toxicity grades | | P value | OR (95% CI) |
|------|---------|----------|--------|----------|---------------|-----------------|--------------------|---------|-------------|
| | | | | | | 0–2, n (%) | 3–4, n (%) | | |
| *DPYD* | c.496A > G | 12 | Met166Val | AA | Dominant | 37 (71) | 15 (29) | | Reference |
| | | | | AG | | 4 (33) | 8 (67) | **0.021** | 4.93 (1.29, 18.87) |
| *DPYD* | c.1129-5923C > G | 2 | splice site | CC | Dominant | 39 (64) | 22 (36) | | Reference |
| | | | | CG | | 2 (67) | 1 (33) | 1.000 | 0.89 (0.08, 10.34) |
| *DPYD* | c.1896 T > C | 4 | synonymous | TT | Dominant | 40 (68) | 19 (32) | | Reference |
| | | | | TC | | 1 (20) | 4 (80) | 0.052 | 8.42 (0.88, 80.56) |
| hsa-mir-27a | rs895819T > C | 33 | hairpin region | TT/TC | Recessive | 40 (69) | 18 (31) | | Reference |
| | | | | CC | | 1 (17) | 5 (83) | **0.020** | 11.11 (1.21, 102.09) |
| *UGT1A1* | *28 | 31 | transcriptional activity | *1*1/ *1*28 | Recessive | 40 (68) | 19 (32) | | Reference |
| | | | | *28*28 | | 1 (20) | 4 (80) | 0.052 | 8.42 (0.88, 80.56) |

Variants in *DPYD* are listed in order of location on the gene. *Minor Allele Frequency in European Individuals (1000 Genome).

rs895819 of hsa-mir-27a showed a significant association with severe toxicity (*P* = 0.020), with an OR of 11.11 (95% CI 1.21, 102.09). Patients with *UGT1A1*28* in homozygous status showed a non-significant trend for association with the occurrence of grade 3–4 AEs (OR 8.42, 95% CI 0.88, 80.56; *P* = 0.052). Finally, the influence of SNPs on grade 3–4 chemotherapy-induced AEs was analyzed using a multivariate analysis, as highlighted in Table 3. An independent association with severe toxicity was found only for *DPYD* c.496A > G (*P* = 0.022) and c.1896 T > C (*P* = 0.027), whereas *UGT1A1*28* was quite near the significance level (*P* = 0.054) and hsa-mir-27a rs895819 was not independently associated with severe toxicity in this series (*P* = 0.161).

Treatment protocol modifications were significantly more frequent in patients carrying the variant allele for *DPYD* c. 496>G (*P* = 0.028) and *DPYD* c.1896T>C (*P* = 0.015)as compared with non-carriers.

## Table 3

Multivariate logistic regression analysis and chemotherapy-induced AEs

| Variables | P value | OR (95% CI) |
|-----------|---------|-------------|
| **DPYD c.496A > G** | | |
| AA | | Reference |
| AG | 0.022 | 5.94 (1.29, 27.22) |
| **DPYD c.1896 T > C** | | |
| TT | | Reference |
| TC | 0.027 | 14.53 (1.36, 155.20) |
| **hsa-mir-27a rs895819T > C** | | |
| TT/TC | | Reference |
| CC | 0.161 | 5.73 (0.50, 65.71) |
| **UGT1A1*28** | | |
| *1*1/*1*28 | | Reference |
| *28*28 | 0.054 | 10.84 (0.96, 122.83) |

There was no association between SNPs and time-to-occurrence of toxicity.

## Discussion

In patients with advanced CRC, the FOLFOXIRI regimen achieved significantly superior outcomes as compared with standard doublet chemotherapy in terms of response rate, progression-free survival and overall survival [3]. Initially, triplet chemotherapy with FOLFOXIRI plus bevacizumab gained popularity as conversion strategy in potentially resectable CRC liver metastases [17] or in selected, poor-prognosis patient populations, such as those with BRAF-mutated tumours [18]. However, this strategy of upfront treatment intensification was recently established as a palliative treatment option due to improvement of patients' survival [4]. We demonstrated that oral capecitabine can safely replace 5-FU as the fluoropyrimidine backbone for combination with oxaliplatin and irinotecan (COI regimen) with a manageable toxicity profile [19]. Nevertheless, diarrhoea is the main dose-limiting toxicity of irinotecan and is also increased when using capecitabine as compared with 5-FU [20, 21]. Initial phase III clinical trials using capecitabine and irinotecan at previously recommended doses showed an unacceptable increase of diarrhoea over fully infusional regimens [22, 23]. The use of modified schedules was demonstrated as feasible in several trials [19, 24]. Since increased toxicity of triplet combinations remains an unquestionable issue, patients' management with adequate supportive measures is a priority for clinicians. The rationale use of pharmacogenetics and the personalization of dosing may allow the safe administration of intensive triplet regimens and maximize their therapeutic index [25].

Guidelines regarding the recommendation of pharmacogenetic tests for personalization of fluoropyrimidines and irinotecan treatment have been published. They suggest predictive screening tests for DPD and UGT1A1 deficiency in order to define the starting doses of fluoropyrimidine and irinotecan followed by titration of dose according to drug tolerability and toxicity [5, 11]. Due to the polyallelic mechanisms of DPD deficiency, a comprehensive predictive screening for fluoropyrimidines toxicity is complex. Besides *DPYD* genotyping, alternative strategies include the possibility of assessing directly the enzymatic activity of DPD. However, assays measuring it in PBMCs are not likely suitable in the clinic due to costs and applicability. Genotyping is simpler, less expensive and can be done at any time. To increase its sensitivity multiple functional variants must be tested.

The clinical usefulness of some common *DPYD* variants such as c.496A > G, c.1129-5923C > G and c.1896 T > C is still to be validated. In our study, we identified a significant association of *DPYD* c.496A > G with grade 3–4 AEs in both univariate and multivariate analysis. This variant allele causes an amino acid substitution in the 166 protein position (M166V), which is located in a conserved three dimensional environment in the DPD protein and could be implicated in compromised enzyme function [26]. However, *DPYD* c.496A > G was associated with 5-FU toxicity in some studies [8], but not in others [27, 28]. This conflicting evidence may depend on the retrospective nature of previous series, heterogeneity of patients and tumours, as well as chemotherapy combinations.The deep intronic variant c.1129-5923C > G was significantly associated with severe 5-FU toxicity in a previous study [7]. Since we detected this variant in only three (5%) patients, the lack of association with toxicity may be due to insufficient statistical power and confounding biases. It is noteworthy to point out that a recent and large study failed to show any significant association of this variant with severe toxicity on multivariate analysis [29]. The *DPYD* c.1896 T > C variant was recently associated with the occurrence of neutropenia due to high serum concentrations of 5-FU [6]. We identified the heterozygous status for *DPYD* c.1896 T > C in five patients. Four of them (80%) experienced grade 3–4 AEs. Possibly due to the small sample size, our analysis could only reach a non-significant trend for association with severe toxicity. The same methodological issue may have influenced the results for patients with *UGT1A1*28/*28*. In fact, it is well described that *UGT1A1*28* genotype may help clinicians to individualize better the adequate dosing of irinotecan when a standard schedule is planned [12]. In general, our results suggest that 21 (33%) patients harbouring at least one *DPYD* variant among *DPYD* c.496A > G, *DPYD* c.1896 T > C and *UGT1A1*28/*28* had an increased risk of toxicity following triplet chemotherapy. Thus, identifying these patients

prior to initiating treatment would allow accurate dose selection and better likelihood of treatment completion.

Finally, inter-individual differences in DPD expression may be also due to epigenetic regulation mechanisms. Indeed, variant alleles of rs895819 in the coding region of the hsa-mir-27a hairpin result in a loop region larger than the common hairpin and positively influence mature miR-27a. As a result of this, DPD enzymatic activity was lower in volunteers carrying the rs895819 variant allele [10]. In our study, we investigated for the first time the clinical significance of rs895819 in cancer patients receiving fluoropyrimidine-based chemotherapy. Even if variant *C* allele in homozygous status at rs895819 of hsa-mir-27a was significantly associated with severe toxicity in univariate analysis, an independent influence was not confirmed in multivariate analysis. This may be due to the fact that four out of six patients in our series carried concomitantly the c.496A > G variant and one out of six carried *UGT1A1*28/*28* (Figure 1), suggesting that the *DPYD* genetic control could have prevailed over the DPD epigenetic regulation.

Our study clearly has some limitations. First of all, this is a retrospective biomarker analysis conducted within two study protocols and the limited sample size still poses the challenge of validation studies in larger data sets of patients receiving triplet chemotherapy. The heterogeneity of treatment protocols according to the monoclonal antibody used (cetuximab *vs.* bevacizumab) and the treatment intent (curative *vs.* palliative) may have affected treatment-related toxicities. In fact, a significant difference in terms of grade 3–4 events was observed in patients enrolled in the two studies. Second, we did not investigate SNPs associated with oxaliplatin-induced toxicities. However, severe neurotoxicity, the main dose cumulative toxicity of oxaliplatin, was not observed in our study, probably because the maximum number of treatment cycles was only eight. The value of our study relies on the series of patients treated homogeneously with triplet chemotherapy within the context of two prospective single arm clinical trials. All patients were trial candidates and showed optimal performance status, low median age and liver laboratory tests within the normal ranges, thus excluding a potential impact of clinical variables on severe toxicity. For example, even if neutropenia is a recognized dose-limiting toxicity of irinotecan and oxaliplatin, we did not report a significant rate of myelotoxicity. The risk of neutropenia was certainly lower based on the favourable clinical characteristics of patients. Moreover, the absence of significant neutropenia is typical of our COI regimen, since capecitabine has a lower myelotoxicity than 5-FU and is given for only 5 consecutive days [18].

In conclusion, we considered simultaneously SNPs involved in both irinotecan and fluoropyrimidines metabolism since they are associated with overlapping toxicities, mainly diarrhoea. We analyzed only a selected

pharmacogenetic panel including the most likely genetic and epigenetic DPD regulators in association with the *UGT1A1*28* functional allele. This strategy led us to understand the part of the percentage of toxicity heritability which is missing from the assessment of recommended *DPYD* variants. Our study provides a rational approach to open new windows for investigation. Intensive regimens such as FOLFOXIRI could be reassessed in selected patients populations, such as those with c.496A > G and/or c.1896 T > C variants, within the context of a 1b dose finding study. However, we emphasize the need to validate our results in a prospective large trial to identify pre-emptively patients at highest risk of toxicity who would require initial protocol modifications followed by a dose escalation model. Genome wide association studies and next generation sequencing technologies in large cohorts may strengthen the results we obtained by candidate gene studies and identify new common or rare risk variants. We believe that prospective validation of our results through a pharmacogenetic-driven trial may be extremely helpful for personalization of fluoropyrimidine doses in patients receiving triplet chemotherapy for advanced CRC.

## Competing Interests

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