The Pharmacogenomics Journal (2021) 21:233–242
https://doi.org/10.1038/s41397-020-00203-7

ARTICLE

# Comprehensive pharmacogenetic analysis of *DPYD*, *UGT*, *CDA*, and *ABCB1* polymorphisms in pancreatic cancer patients receiving mFOLFIRINOX or gemcitabine plus nab-paclitaxel

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Received: 20 April 2020 / Revised: 9 November 2020 / Accepted: 4 December 2020 / Published online: 18 January 2021
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## Abstract

Modified FOLFIRINOX (mFOLFIRINOX) and gemcitabine + nab-paclitaxel (GemNab) regimens represent a standard treatment in advanced pancreatic cancer (aPC). DPYD and UGT1A1 variants are relevant predictors of fluoropyrimidine and irinotecan-associated adverse events (AEs). Furthermore, data about the associations between polymorphisms in ABCB and CDA genes and GemNab-related toxicities are still controversial. The present study analyzes the association between DPYD, UGT, ABCB1, CDA variants, and AEs in aPC patients (pts) treated with mFOLFIRINOX or GemNab. Blood samples collected from 104 aPC pts treated with mFOLFIRINOX and 63 with GemNab were tested for DPYD c.1679T>G, IVS14 +1G>A, c.2194G>A, c.2846A>T, UGT1A1*28, CDA c.79A>C, and ABCB1 c.1236C>T, c.2677G>T/A, c.3435C>T by real-time PCR and automatic sequencing. In mFOLFIRINOX cohort, DPYD IVS14+1GA genotype was associated with G4 hematological AEs, while the UGT1A1*28 significantly correlated with the risk of thrombocytopenia ($p = 0.006$). In the GemNab cohort, a significant association between CDA c.79CC and high-grade nausea was observed ($p = 0.002$). Moreover, the presence of at least a mutant allele in ABCB1 increased the risk of overall hematological AEs ($p = 0.01$), both further strengthened by the presence of CDA c.79CC ($p = 0.0002$). DPYD IVS14+1A allele is confirmed to be associated with fluoropyrimidine life-threatening toxicities, and UGT1A1*28 is related with a higher risk of hematologic AEs following irinotecan treatment. CDA c.79C and ABCB1 c.1236T, c.2677T/A, and c.3435T mutant alleles are predictive biomarkers of GemNab-related AEs. All these variants should be considered in aPC pts candidate to mFOLFIRINOX or GemNab treatments.

## Introduction

Pancreatic cancer is the 4th leading cause of cancer-related deaths in US [1] and trends for pancreatic cancer mortality are rising both in US and in Europe [2].

Systemic chemotherapy represents the standard of care in advanced disease and, based on the efficacy results obtained from randomized trials, two regimens are currently recommended as first-line treatment: the combination of 5-fluorouracil (5FU), oxaliplatin and irinotecan (FOLFIRINOX) and the combination of gemcitabine and nab-paclitaxel (GemNab), both able to improve patients' outcome compared to gemcitabine monotherapy [3, 4]. Recently, adjuvant treatment with modified FOLFIRINOX (mFOLFIRINOX) resulted in better overall survival (OS) and disease-free survival (DFS) compared to gemcitabine

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**Supplementary information** The online version of this article (https://doi.org/10.1038/s41397-020-00203-7) contains supplementary material, which is available to authorized users.

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monotherapy; for this reason, mFOLFIRINOX represents the new standard of care also for resected patients [5].

Both FOLFIRINOX and GemNab are associated with relevant toxicities, especially neutropenia, thrombocytopenia, and diarrhea [3, 4], although different clinical factors such as age, sex and comorbidities could influence treatment compliance [6, 7]. Importantly, a decreased function in enzymes involved in drug metabolism could play a major role in developing treatment-related AEs.

The metabolism of irinotecan and 5-FU involves two liver enzymes, uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) and dihydropyrimidine dehydrogenase (DPD), respectively [8, 9]. The UGT1A1*28 polymorphism is characterized by an increased number of the [TA] repeated sequence in the TATA region of the UGT1A1 promoter (6 [TA] repetitions represent the wild-type condition (*1/*1) and 7 repetitions are the mutant condition *28/*28). The [TA] increase is related to a reduced UGT1A1 expression, therefore, the amount of metabolized irinotecan decreases allowing an increased risk of toxicity. It has been demonstrated that homozygous and heterozygous patients for UGT1A1*28 presented higher grades of diarrhea and neutropenia as a result of poor-metabolizer phenotype [10, 11]. Similarly, DPD activity is strongly reduced in presence of one of the following four mutations: IVS14+1G>A (rs3918290, DPYD*2A), c.1679T>G (rs55886062, DPYD*13), c.2846A>T (rs67376798), and c.1129-5923C>A (rs75017182, c.1236G>A/HapB3), proving to be relevant clinical predictors of fluoropyrimidine-associated toxicity [12, 13]. Interestingly, the association between DPD polymorphism c.2194G>A and clinically significant AEs in cancer patients treated with fluoropyrimidines has been also demonstrated [14–17].

Regarding the GemNab regimen, several studies evaluated the presence of single nucleotide polymorphisms (SNPs) in the ATP Binding Cassette Subfamily B Member (ABCB transporters), and in the cytidine deaminase (CDA) genes in patients treated with gemcitabine or taxanes-based chemotherapy and developing severe AEs [18–21] with controversial results. Polymorphisms in ABCB gene have been reviewed and correlated with diverse expression of efflux pumps in several tissue compartments and, as a result, modified drug levels [22]. Chang et al. demonstrated an association between ABCB1 polymorphisms (c.2677G/T>A and c.3435C>T) and toxicity (mucositis and diarrhea) in advanced gastric cancer patients treated with paclitaxel-based chemotherapy [23]. Furthermore, ABCB1 polymorphisms have been related to hematological AEs in cancer patients receiving taxanes [21, 24–28]. CDA is involved in the metabolic inactivation of gemcitabine [29], and the c.79A>C polymorphism has been reported to cause a reduction in CDA enzyme activity, allowing a reduced metabolism of gemcitabine and consequently increased risk of AEs [30]. In the phase III Radiation Therapy Oncology Group (RTOG) 9704 study evaluating the addition of gemcitabine to pre- and post- chemoradiation with 5FU in resected pancreatic cancer patients, the presence of CDA c.79A>C polymorphism was associated with gemcitabine hematological toxicity [19]. Similarly, in a retrospective analysis, CDA c.79A>C was correlated with grade 3–4 neutropenia in 149 advanced pancreatic cancer patients who underwent gemcitabine-based chemoradiotherapy [18]. In contrast, the analysis lead by Okazaky et al. did not show any association between CDA c.79A>C and the AEs of gemcitabine-based chemoradiotherapy in pancreatic cancer patients [31].

The present study is aimed to evaluate the role of known polymorphisms in DPYD, UGT, ABCB, and CDA genes and their possible association with toxicity in pancreatic cancer patients treated with modified FOLFIRINOX (mFOLFIRINOX) [32] or GemNab.

## Patients and methods

### Patients' characteristics

Patients affected by advanced pancreatic adenocarcinoma and treated at the University Hospital of Pisa with first-line mFOLFIRINOX or gemcitabine + nab-paclitaxel between 2010 and 2017 were enrolled. All patients were treated with standard schedule and dose reductions were performed in case of toxicity as per clinical practice. Administered schedule for mFOLFIRINOX was as follows: oxaliplatin 85 mg/sqm intravenously on day 1, irinotecan 165 mg/sqm intravenously on day 1, leucovorin 200 mg/sqm intravenously on day 1, fluorouracil 3200 mg/sqm 48 h continuous infusion starting on day 1, cycles repeated every 14 days. Administered schedule for gemcitabine + nab-paclitaxel was as follows: gemcitabine 1000 mg/sqm intravenously on days 1, 8, and 15 + nab-paclitaxel 12 mg/sqm on days 1, 8, and 15, cycles repeated every 28 days.

Main inclusion criteria were: availability of data regarding toxicity and efficacy of first-line treatment, a minimum of 1 administered completed chemotherapy cycle, and availability of blood sample.

The study was approved by the Ethics Committee of Pisa University Hospital and conducted in accordance with the principles of the Declaration of Helsinki; all patients gave their signed informed consent before blood collection and DNA analysis.

Laboratory investigators were blinded to the clinical information on patients' outcome.

### Methods

Three ml of whole blood were taken from all patients, stored in EDTA, and genomic DNA was extracted from



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200 µl by the Biorobot EZ1 (Qiagen®, Valencia, CA). The analysis of DPYD, CDA and ABCB1 variants were conducted on a Real-Time PCR instrument (HT 7900 Life Technologies, Carlsbad, CA), by using the TaqMan® SNP Genotyping Assay. UGT1A1*28 variant was evaluated on a Sanger Sequencing 3100 instrument (Life Technologies, Carlsbad, CA). Analyzed variants included: DPYD c.1679T>G, IVS14+1G>A, c.2194G>A, c.2846A>T; UGT1A1*28; CDA c.79A>C; ABCB1 c.1236C>T, c.2677G>T/A, c.3435C>T. Negative, positive controls, and blanks were used to assess the genotype of each variant analyzed.

## Statistical analysis

Toxicity during treatment was graded by the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.03. Categorical data were described by absolute and relative frequencies, whereas quantitative data were reported as mean and standard deviation. The frequencies of DPYD, UGT1A1, CDA, and ABCB1 polymorphisms were compared to the worldwide population, and Hardy–Weinberg equilibrium was assessed.

The association between each gene polymorphism and AEs was evaluated by chi-square test, adjusting the $p$ value by the appropriate $z$-test for proportions. Odds ratio was also calculated. A significant threshold of 0.025 was considered for DPYD c.2194G>A and UGT1A1*28 analysis, after Bonferroni correction. Likewise, Bonferroni post hoc test for multiple testing of 2 genes (CDA, ABCB1) was applied, giving a significant threshold of 0.025 (=0.05/2). Applying this across the common variants tested ($n = 4$) would give a Bonferroni-corrected $p$ value of 0.0125.

Mann–Whitney analysis was used to evaluate the different grades of AEs and SNPs. Progression-free survival (PFS) and OS were defined as the time from treatment start to progression disease or death, respectively. The Kaplan–Meier method was used to create PFS curves and log-rank test was used to evaluate the differences between curves.

Statistical significance was defined by a $p$ value $< 0.05$. Analysis was performed by MedCalc version 14.8.1 and IBM SPSS statistics for Windows, version 26.0 (Armonk, NYI).

# Results

A total 167 patients were consecutively enrolled in this study and divided in 2 cohorts, depending on the treatment received; 104 patients were treated with mFOLFIRINOX and 63 with GemNab. Main clinical characteristics of the whole population are reported in Table 1. The calculation of Hardy–Weinberg equilibrium was performed; nevertheless, not all the polymorphisms were found to be in equilibrium (Table 2).

**Table 1** Patient characteristics.

| | All patients | | Cohort 1 (mFOLFIRI-NOX) | | Cohort 2 (GemNab) | |
|---|---|---|---|---|---|---|
| Characteristics | $N = 167$ | % | $N = 104$ | % | $N = 63$ | % |
| Age years, median (range) | 64 (44–77) | | 63 (44–75) | | 65 (45–77) | |
| Gender | | | | | | |
| Male | 82 | 49 | 51 | 49 | 31 | 49 |
| Female | 85 | 51 | 53 | 51 | 32 | 51 |
| ECOG performance status | | | | | | |
| 0 | 115 | 69 | 83 | 80 | 32 | 51 |
| 1 | 49 | 29 | 20 | 19 | 29 | 46 |
| 2 | 3 | 2 | 1 | 1 | 2 | 3 |
| Previous surgery on primary tumor | | | | | | |
| Yes | 31 | 19 | 16 | 15 | 15 | 24 |
| No | 136 | 81 | 88 | 85 | 48 | 76 |
| Tumor stage | | | | | | |
| III | 44 | 26 | 41 | 39 | 3 | 5 |
| IV | 123 | 74 | 63 | 61 | 60 | 95 |
| Number of metastatic sites median (range) | 1 (1–4) | | 2 (1–5) | | 2 (1–4) | |
| Metastatic sites | | | | | | |
| Liver | 88 | 53 | 47 | 45 | 41 | 63 |
| Lung | 18 | 11 | 7 | 7 | 11 | 17 |
| Lymph nodes | 37 | 22 | 23 | 22 | 14 | 22 |
| Peritoneum | 34 | 20 | 17 | 16 | 17 | 27 |
| Local recurrence | 5 | 3 | 2 | 2 | 3 | 5 |
| Other | 7 | 4 | 4 | 4 | 3 | 5 |
| Number of administered cycles median (range) | – | | 8 (1–27) | | 4 (1–19) | |

## Cohort 1

In the mFOLFIRINOX cohort, 53 (51%) patients experienced grade ≥3 AEs, with 44 (42%) patients reporting hematologic and 20 (19%) patients reporting gastrointestinal toxicities, respectively. Main grade >2 AEs were neutropenia (40.4%), diarrhea (7.7%), and stomatitis (6.7%). In fifty-one patients (49%) at least one cycle of chemotherapy was postponed and 39 (37%) patients needed a dose reduction.

The DPYD analysis found one patient carrier of the IVS14+1GA genotype, 8 patients had the c.2194GA genotype, while no carriers of c.1679T>G or c.2846A>T were found. At the UGT analysis, 38 patients were UGT1A1*1/*28, while 18 patients were UGT1A1*28/*28. Additionally, three patients showed the concomitant presence of DPYD c.2194GA, and UGT1A1*1/*28 genotype.


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**Table 2** Frequency of DPYD, UGT1A1, CDA, and ABCB1 SNPs.

| SNPs | Genotypes | *N* | (%) | HWE ($p_{HWE}$) |
|---|---|---|---|---|
| DPYD c.2194G>A | GG | 96 | 92 | 0.17 (0.68) |
| | GA | 8 | 8 | |
| | AA | – | – | |
| UGT1A1*28 | *1/*1 | 48 | 46.2 | 4.28 (0.04)[a] |
| | *1/*28 | 38 | 36.5 | |
| | *28/*28 | 18 | 17.3 | |
| CDA c.79A>C | AA | 18 | 28.6 | 0.39 (0.53) |
| | AC | 29 | 46 | |
| | CC | 16 | 25.4 | |
| ABCB1 c.1236C>T | CC | 16 | 25.4 | 0.15 (0.69) |
| | CT | 33 | 52.4 | |
| | TT | 14 | 22.2 | |
| ABCB1 c.2677G>T/A | GG | 18 | 28.6 | 9.43 (0.002)[a] |
| | GT | 19 | 30.1 | |
| | TT + TA | 26 | 41.3 | |
| ABCB1 c.3435C>T | CC | 17 | 27 | 0.40 (0.53) |
| | CT | 29 | 46 | |
| | TT | 17 | 27 | |

*SNPs* single nucleotide polymorphisms, *N* number of patients.
[a]Disequilibrium of HWE.

The statistical analysis for the IVS14+1GA was not performed since only one patient was carrier of the mutant allele; however, the patient experienced G4 hematological (febrile neutropenia and thrombocytopenia) and G3 non-hematological (mucositis) AEs after the first cycle at a standard dose and treatment was interrupted.

Three out of five patients harboring c.2194GA genotype and wild-type UGT experienced at least one grade >2 AEs during treatment and the most frequent grade >2 AEs were neutropenia (60%) and stomatitis (20%). Twenty-seven out of 53 patients (51%) bearing UGT1A1*28 (hetero or homozygous condition) with a wild-type DPYD genotype experienced at least one grade >2 AE during treatment and most frequent grade >2 AEs were neutropenia (43%) and stomatitis (8%). Two out of the three patients showing the concomitant presence of DPYD c.2194GA and UGT1A1*1/*28 genotype experienced grade >2 AE (neutropenia).

No significant association between c.2194GA and any grade of hematologic ($p = 0.08$ for thrombocytopenia risk) and gastrointestinal 5-FU related toxicity or hand–foot syndrome (HFS; $p = 0.096$) was found.

Regarding irinotecan, the association between UGT1A1*28 and any grade (or grade ≥ 3) of hematologic or gastrointestinal toxicities was evaluated. The UGT1A1*28 allele was significantly correlated with the risk of developing thrombocytopenia (OR 3.17, 95% CI 1.27–7.93, $p = 0.006$). Moreover, stratifying patients based on the presence of mutant alleles and their risk of developing thrombocytopenia, the *28/*28 or *1/*28 genotypes were at increased risk with respect to the *1/*1 (i.e., the risk of thrombocytopenia decreased with this order: *28/*28 > *1/*28 > *1/*1, $p = 0.003$). Grouping patients based on their haplotypes, including DPYD and UGT, a significant association in the subgroup of patients carrying a wild-type DPYD and UGT1A1*1/*28 was found with gastrointestinal toxicities ($p = 0.004$; Table 3).

In terms of efficacy, no difference in PFS (Hazard Ratio 0.81, 95% confidence interval 0.47–1.40, $p = 0.45$) and OS (HR 1.22, 95% CI 0.67–2.27, $p = 0.51$) were reported in patients harboring *28 alleles who underwent dose reduction ($N = 26$) compared to wild-type patients treated with standard dose who did not need dose adjustments ($N = 33$) (Fig. 1).

Sixteen out of 104 patients in the FOLFIRINOX cohort undergone resection of primary tumor prior to chemotherapy starting. None of them was carrier of a DPYD polymorphism, while ten patients harbored the UGT variant (four were UGT1A1*1/*28 and six were UGT1A1*28/*28). Incidence of diarrhea and high-grade diarrhea did not significantly differ between resected and not-resected patients ($p = 0.74$ and $p = 0.33$, respectively). In resected patients no significant association between UGT polymorphisms and any grade (or grade ≥ 3) diarrhea was found ($p = 0.95$ and $p = 0.69$, respectively).

## Cohort 2

In the GemNab cohort, 36 (57%) patients experienced at least a grade ≥ 3 AEs, with 35 (56%) patients reporting hematologic toxicities and 2 (3%) patients reporting gastrointestinal toxicities, respectively. Main grade >2 AEs were neutropenia (48%), anemia (17%) and thrombocytopenia (13%). In forty-two patients (67%) at least one cycle of chemotherapy has been postponed and in 18 (29%) dose of chemotherapy was reduced due to AEs.

The analysis of the CDA c.79A>C polymorphism discovered 29 heterozygous and 16 homozygous patients. The analysis of ABCB1 identified a total of 47 mutant patients; in detail, 33 heterozygous and 14 homozygous patients for the c.1236C>T SNP; 19 heterozygous and 26 homozygous patients for the c.2677G>TA polymorphism and 29 heterozygous and 17 homozygous patients for the c.3435C>T SNP. Thirty-six patients showed concomitant presence of CDA and ABCB1 variants. In detail, CDA c.79AC and c.79CC variants were concomitantly found with ABCB1 c.1236CT/TT, c.2677GT/AT/TT, and c.3435CT/TT in 23 and 13 patients, respectively.

Grouping patients based on their haplotypes, including CDA and ABCB1 together, a significant association



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**Table 3** Associations of mFOLFIRINOX toxicity and candidate SNPs by grouping for concomitant polymorphisms.

| Genotype | Non-hematologic toxicities | Nausea (yes vs. no) *p* value | Nausea ≥ G3 (no vs. yes) *p* value | Vomiting (no vs. yes) *p* value | Vomiting ≥ G3 (no vs. yes) *p* value | Diarrhea (yes vs. no) *p* value | Diarrhea ≥ G3 (no vs. yes) *p* value | Stomatitis (yes vs. no) *p* value | Stomatitis ≥ G3 (no vs. yes) *p* value | Hand–foot syndrome (no vs. yes) *p* value | Over-all (yes vs. no) *p* value | Overall ≥ G3 (no vs. yes) *p* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| c.2194GG UGT1A1$_{*1/*1}$ | | 0.94 | 0.19 | 0.42 | 0.77 | 0.99 | 0.19 | 0.77 | 0.45 | 0.94 | 0.09 | 0.16 |
| c.2194GG UGT1A1*1/*28 | | 0.94 | 0.37 | 0.55 | 0.32 | 0.63 | 0.79 | 0.73 | 0.13 | 0.84 | **0.004** | 0.64 |
| c.2194GG UGT1A1*28/*28 | | 0.74 | 0.92 | 0.06 | 0.26 | 0.59 | 0.16 | 0.76 | 0.18 | 0.18 | 0.33 | 0.05 |
| c.2194GA UGT1A1*1/*1 | | 0.25 | 0.62 | 0.13 | 0.73 | 0.89 | 0.54 | 0.54 | 0.17 | 0.17 | 0.67 | 0.92 |
| c.2194GA UGT1A1*1/*28 | | 0.31 | 0.62 | 0.27 | 0.79 | 0.84 | 0.60 | 0.19 | 0.62 | 0.08 | 0.71 | 0.34 |
| **Genotype** | **Hematologic toxicities** | **Neutropenia (yes vs. no) *p* value** | **Neutropenia ≥ G3 (no vs. yes) *p* value** | **Thrombocytopenia (no vs. yes) *p* value** | **Thrombocytopenia ≥ G3 (no vs. yes) *p* value** | **Anemia (yes vs. no) *p* value** | **Anemia ≥ G3 (no vs. yes) *p* value** | **Overall (yes vs. no) *p* value** | **Overall ≥ G3 (yes vs. no) *p* value** | | | |
| c.2194GG UGT1A1*1/*1 | | 0.31 | 0.27 | **0.003** | 0.80 | 0.67 | 0.98 | 0.41 | 0.82 | | | |
| c.2194GG UGT1A1*1/*28 | | 0.96 | 0.67 | 0.20 | 0.21 | 0.32 | 0.75 | 0.16 | 0.83 | | | |
| c.2194GG UGT1A1*28/*28 | | 0.62 | 0.94 | 0.42 | 0.39 | 0.35 | 0.94 | 0.63 | 0.56 | | | |
| c.2194GA UGT1A1*1/*1 | | 0.74 | 0.22 | 0.10 | 0.71 | 0.52 | 0.63 | 0.83 | 0.44 | | | |
| c.2194GA UGT1A1*1/*28 | | 0.22 | 0.45 | 0.26 | 0.75 | 0.20 | 0.68 | 0.86 | 0.71 | | | |

*SNPs* single nucleotide polymorphisms, *G* grade.

Values in bold are significant.



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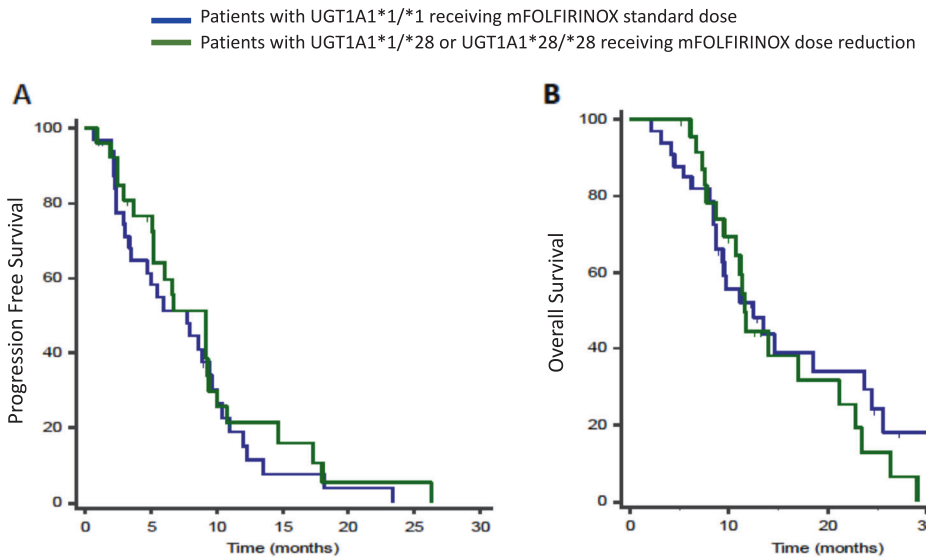



**Fig. 1 Involvement of UGT1A1*28 in clinical outcome of patients.** Progression-free survival (**A**) and overall survival (**B**) in patients harboring UGT1A1*28 alleles who needed dose reduction vs. wild-type patients treated with standard dose mFOLFIRINOX.

between CDA c.79CC and high-grade nausea was observed in the subgroup of patients carrying the CDA mutant genotype and wild-type ABCB1 ($p = 0.002$; Table 4). Moreover, patients with a heterozygous or homozygous variant for one of the ABCB1 SNPs had an increased risk of hematological toxicities, including anemia ($p = 0.01$; Table 4), and the presence of CDA c.79CC strengthened these significant associations ($p = 0.0002$; Table 4). Comprehensive statistical analysis is reported in Supplementary Table 1.

## Discussion

The role of DPYD and UGT polymorphisms in fluoropyrimidine and irinotecan-correlated toxicity was initially investigated in colorectal cancer [14, 33]. In pancreatic cancer, an exploratory analysis of the observational JASPAC 06 trial investigated the correlation between the incidence of AEs in Japanese patients treated with FOLFIRINOX and UGT1A1*6 and *28 variant alleles. In this study, patients with UGT1A1 heterozygous genotype experienced severe toxicity (i.e., diarrhea) compared with patients without UGT1A1*6 or *28 alleles [34].

Recently, a case report described a 59-year-old metastatic pancreatic cancer patient receiving FOLFIRINOX therapy showing a severe 5-FU toxicity after a single cycle. Interestingly, the sequencing on entire DPYD gene revealed heterozygous genotypes for three different polymorphisms: DPYD*2A (IVS14+1G>A), c.1601G>A and c.2194G>A. The patient was also carrier of the UGT1A1*28/*28 genotype [35]. Similarly, Velez-Velez et al. presented a case of a patient with pancreatic adenocarcinoma who was found homozygous for the UGT1A1*28 allele, and heterozygous for a DPYD variant through pharmacogenomic testing. In this case, severe toxicity was prevented by a reduction of starting dose, and these results suggested the clinical value of integrating pharmacogenomic testing into clinical practice to allow a preemptive dose reduction in patients at high risk of toxicity [36].

Our study confirms the association between DPYD IVS14 +1A allele with life-threatening toxicities. While other published studies demonstrated an association between c.2194G>A and fluoropyrimidine-related toxicity [37, 38], we were unable to find significant correlations, probably due to the small number of c.2194GA allele carriers.

The clinical impact of the c.2194G>A SNP still need to be confirmed prospectively; however, published retrospective studies showed a correlation with fluoropyrimidine-related toxicity [15, 16, 39, 40]. The TOSCA trial confirmed the association between c.2194G>A and fluoropyrimidine-related toxicity in 508 colorectal cancer patients treated with FOLFOX or XELOX adjuvant chemotherapy for 3 or 6 months [16]. In a subgroup analysis carried out from the CAIRO trial, the c.2194G>A was significantly associated with grade 3–4 diarrhea, even if with a low predictive value (41%) [40]. An association between the c.2194G>A and neutropenia was also found in the PETACC-8 trial, which enrolled 1545 patients affected by colorectal cancer, receiving standard adjuvant FOLFOX4 or FOLFOX4 in combination with cetuximab [15]. A recent published study on 366 fluoropyrimidines-treated patients confirmed the association between the c.2194G>A and severe neutropenia [17].



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**Table 4** Significant associations of GemNab AEs and candidate SNPs by grouping for co-occurring polymorphisms. Bonferroni post-hoc test was applied across the common CDA and ABCB1 variants ($n = 4$) giving a significant threshold of 0.0125 (=0.05/4).

| Genotype | Non-hematologic toxicities | Nausea (yes vs. no) *p* value | Nausea ≥ G3 (yes vs. no) *p* value | Vomiting (yes vs. no) *p* value | Diarrhea (no vs. yes) *p* value | Diarrhea ≥ G3 (yes vs. no) *p* value | Stomatitis (no vs. yes) *p* value | Overall (yes vs. no) *p* value | |
|---|---|---|---|---|---|---|---|---|---|
| CDA c.79AA<br>ABCB1 c.1236$_{CT}$<br>ABCB1 c.2677$_{GG}$<br>ABCB1 c.3435$_{CT}$ | | 0.27 | 0.86 | 0.56 | 0.37 | 0.86 | 0.51 | 0.05 | |
| CDA c.79AC<br>ABCB1 c.1236$_{CC}$<br>ABCB1 c.2677$_{GG}$<br>ABCB1 c.3435$_{CC}$ | | 0.80 | 0.64 | 0.59 | 0.23 | 0.05 | 0.26 | 0.19 | |
| CDA c.79AC<br>ABCB1 c.1236$_{TT}$<br>ABCB1 c.2677$_{TT/TA/AA}$<br>ABCB1 c.3435$_{TT}$ | | 0.02 | 0.05 | 0.02 | 0.23 | 0.64 | 0.26 | 0.19 | |
| CDA c.79CC<br>ABCB1 c.1236$_{CC}$<br>ABCB1 c.2677$_{GG}$<br>ABCB1 c.3435$_{CC}$ | | 0.67 | **0.002** | 0.10 | 0.71 | 0.75 | 0.35 | 0.37 | |
| CDA c.79CC<br>ABCB1 c.1236$_{CT}$<br>ABCB1 c.2677$_{GT/GA}$<br>ABCB1 c.3435$_{CT}$ | | 0.54 | 0.64 | 0.13 | 0.73 | 0.05 | 0.45 | 0.42 | |
| CDA c.79CC<br>ABCB1 c.1236$_{CT}$<br>ABCB1 c.2677$_{TT/TA/AA}$<br>ABCB1 c.343$_{CT}$ | | 0.11 | 0.79 | 0.40 | 0.21 | 0.79 | 0.35 | **0.01** | |
| | **Hematologic toxicities** | **Neutropenia (yes vs. no) *p* value** | **Neutropenia ≥ G3 (no vs. yes) *p* value** | **Thrombocytopenia (yes vs. no) *p* value** | **Thrombocytopenia ≥ G3 (no vs. yes) *p* value** | **Anemia (yes vs. no) *p* value** | **Anemia ≥ G3 (no vs. yes) *p* value** | **Overall (yes vs. no) *p* value** | **Overall ≥ G3 (yes vs. no) *p* value** |
| CDA c.79AA<br>ABCB1 c.1236$_{CC}$<br>ABCB1 c.2677$_{GG}$<br>ABCB1 c.3435$_{CT}$ | | 0.16 | 0.34 | 0.35 | 0.70 | 0.85 | 0.04 | 0.85 | 0.38 |
| CDA c.79AA<br>ABCB1 c.1236$_{CT}$<br>ABCB1 c.2677$_{GT/GA}$<br>ABCB1 c.3435$_{CT}$ | | 0.50 | 0.05 | 0.23 | 0.42 | **0.01** | 0.30 | **0.01** | 0.02 |
| CDA c.79AA<br>ABCB1 c.1236$_{CT}$<br>ABCB1 c.2677$_{TT/TA/AA}$<br>ABCB1 c.3435$_{CC}$ | | 0.47 | 0.29 | 0.35 | 0.70 | 0.85 | 0.04 | 0.85 | 0.38 |
| CDA c.79AC<br>ABCB1 c.1236$_{CC}$<br>ABCB1 c.2677$_{GG}$<br>ABCB1 c.3435$_{CT}$ | | 0.16 | 0.34 | 0.35 | 0.70 | 0.84 | 0.04 | 0.85 | 0.38 |
| CDA c.79CC<br>ABCB1 c.1236$_{CT}$<br>ABCB1 c.2677$_{TT/TA/AA}$<br>ABCB1 c.343$_{CT}$ | | 0.05 | 0.17 | 0.90 | 0.58 | **0.0002** | 0.47 | **0.0002** | 0.09 |

Values in bold are significant.



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Concerning UGT, the presence of 1A1*28 allele was significantly associated with AEs, including thrombocytopenia and neutropenia, as also reported in literature [10, 11, 41].

With regards to GemNab treatment, the present study evaluated the role of polymorphisms in CDA and ABCB1 genes and their relationship with toxicity development. Previous analyses independently investigated this association in patients affected by solid tumor—including pancreatic cancer—with controversial results [18–21, 23, 42–45].

A multicenter prospective clinical trial in gemcitabine-treated pancreatic patients failed to identify CDA status as predictive marker of toxicities [45]. Similarly, a study conducted by Chang et al. did not show a correlation between ABCB1 genotypes and paclitaxel AEs [44]. In the present study, the combined presence of heterozygous or homozygous variants for CDA c.79A>C and ABCB1 significantly correlated with gastrointestinal and hematological AEs. A meta-analysis [46] suggested that CDA c.79A>C may be a potential genotype marker of gemcitabine-related severe anemia, in agreement with our findings. Similarly, Ding et al. suggested that CDA c.79C allele is associated with high-grade hematological AEs and reduced CDA activity [20].

The present study considered the most frequent CDA/ABCB1 variants for Caucasian populations [47, 48]. Nonetheless, other variants have been considered in other studies, according to the ethnicity. As an example, the screening for CDA c.208G>A polymorphism may have a clinical meaning in Asian and Japanese population treated with gemcitabine, being reported as a major cause of life-threatening toxicities [49–51]. No other evidence emerged in literature for ABCB1 gene. Despite the present study has some limitations, including its retrospective nature, it provides one of the first extensive pharmacogenetic characterization of pancreatic cancer patients treated with FOLFIRINOX or GemNab regimens and it would be certainly interesting to evaluate the possible correlation between SNPs and AEs in a larger validation prospective cohort, to develop a genotype-guided approach for treatment personalisation.

Given the difficult management and the poor outcome of pancreatic cancer, the improvement in tolerability represents an essential issue both in advanced and in early stages. To date, several recommendations suggest the preemptive screening of the DPYD and UGT1A1*28 variants, suggesting dose adjustment in poor metabolizers [52, 53].

Recently, EMA's safety committee has recommended that patients should be tested for DPD deficiency before starting treatment with fluorouracil, capecitabine, and tegafur [54].

The Italian Association of Medical Oncology and the Italian Society of Pharmacology (AIOM-SIF working group) released a national recommendation for DPYD and UGT screening, including also the c.2194G>A variant [55]. The suggested dose reduction in patients harboring the IVS14+1GA, c.1679TG, 2846AT genotypes is 50%, and for homozygous patients a fluoropyrimidines-based treatment is not suggested. A dose reduction of 15% or 30% in the presence of heterozygous or homozygous c.2194G>A genotypes, respectively, is suggested. A recent meta-analysis demonstrated the significant relationship between UGT1A1*28 polymorphism with toxicity and response to irinotecan-based chemotherapy [56]. However, our data demonstrate that in the presence of a deficient genotype, the response to treatment is not reduced in case of dose adjustment.

In order to prevent the occurrence of treatment-related AEs in pancreatic cancer patients carrying DPYD or UGT1A1*28 variants, a screening test prior to start FOLFIRINOX regimen is therefore required.

For patients with at least one mutated allele of UGT1A1, careful management of hematological and gastrointestinal toxicity is recommended, and a modified FOLFIRINOX with a dose reduction of 30% of irinotecan in homozygous patients might be appropriate.

In conclusion, no upfront genotyping test seems to predict AEs in pancreatic cancer patients treated with Gem-Nab. Further studies with larger validation cohorts are needed to confirm the possible influence of CDA and ABCB1 polymorphisms on Gem-Nab related toxicities. Conversely, in accordance with literature data, our results support DPYD and UGT1A1 genotype-guided dosing schedules for the management of patients candidate to FOLFIRINOX regimen.

## Data availability

Data and results are available at the Unit of Clinical Pharmacology and Pharmacogenetics, University Hospital of Pisa.

**Funding** This work was supported by a grant of the University of Pisa (Italy) to RD.

**Author contributions** Conception and design: MDR, CV. Development of methodology: MDR, SC, ER, and EA. Clinical protocols/amendments: MDR, RD. Acquisition of data: MDR, SC, ER, EA, and CV. Analysis and interpretation of data: MDR, CV, SC, FC. RM, and RD. Writing, review, and/or revision of the paper: all authors. Administrative, technical, or material support: MDR, SC, ER, and RD. Study supervision: MDR, RD.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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**Ethical approval and consent to participate** The study was approved by the Ethics Committee of Pisa University Hospital. Written informed consent was obtained from each patient included, and this study was performed in accordance with the Declaration of Helsinki.

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