A case report of a severe fluoropyrimidine-related toxicity due to an uncommon DPYD variant

Vincenzo De Falco, MD, Maria Iole Natalicchio, PhD, Stefania Napolitano, MD, Nicola Coppola, MD, PhD, Giovanni Conzo, MD, Erika Martinelli, MD, PhD, Nicoletta Zanaletti, MD, Pasquale Vitale, MD, Emilio Francesco Giunta, MD, Maria Teresa Vietri, PhD, Pietro Paolo Vitiello, MD, Davide Ciardiello, MD, Anna Marinaccio, PhD, Ferdinando De Vita, MD, PhD, Fortunato Ciardiello, MD, PhD, Teresa Troiani, MD, PhD.

Abstract

Introduction: Fluoropyrimidines such as 5-fluouracil (5-FU) and its orally active prodrug, capecitabine, are widely used in the treatment of gastrointestinal cancer, including colorectal cancer. Dihydropyrimidine dehydrogenase (DPD) plays an important role in the 5-FU metabolism. Dihydropyrimidine dehydrogenase gene (DPYD) is a highly polymorphic gene with several hundreds of reported genetic variants and DPD activity levels vary considerably among individuals, with different 5-FU-related efficacy and toxicity. About 5% of the population is deficient in DPD enzyme activity. The most well studied DPYD variant is the IVS14+1G>A, also known as DPYD *2A. In this report, we present a case of a patient with a double heterozygote DPYD variant (DPYD activity score: 0.5 according to Clinical Pharmacogenetics Implementation Consortium) who experienced a severe fluoropyrimidine-related toxicity resolved without any consequence.

Patient concerns: A 46-years-old Caucasian man with diagnosis of left colon adenocarcinoma underwent left hemicolectomy on July 2017: pT3 G3 N1c M0. According to the disease stage, he started an adjuvant therapy with XELOX using capecitabine at 50% of total dose, because of his DPYD IVS14+1G>A variant, detected before the treatment. DIAGNOSIS: After few days, despite of this dose reduction, he experienced life-threatening adverse events such as mucositis G3, diarrhea G3, neutropenia G4, thrombocytopenia G4, and hyperbilirubinemia G3 according to Common Terminology Criteria for Adverse Events v 5.0.

Interventions: As first, we set up an intensive rehydration therapy, antibiotic and antifungal prophylaxis, Granulocyte-Colony Stimulating Factors, and supportive blood transfusions. Additional genetic tests revealed a double heterozygote variant of DPYD gene (DPYD IVS14+1G>A and 2846A>T) which is a very rare situation and only 3 cases are described in literature, all of them concluded with patient’s death.

Outcomes: After 3 weeks of intensive therapy, the patient was fully recovered. Furthermore, all the whole-body CT scans performed since discharge from the hospital until now, have confirmed no evidence of disease.

Conclusions: Recent studies demonstrated that screening strategy for the most common DPYD variants allowed for avoiding toxicities and saving money. This report underlines the importance of genotyping DPYD before treatment and emphasizes the role of genotype-guided dose individualization.

Abbreviations: 5,10-MTHF = 5,10-Methylentetrahydrofolate, 5-FU = 5-fluorouracil, AEs = Adverse events, CDA = cytidine deaminase, CES = carboxylesterase, CPIC = Clinical Pharmacogenetics Implementation Consortium, CRC = colorectal cancer, CT = computed tomography, CTCAE = Common Terminology Criteria for Adverse Events, DPD = dihydropyrimidine dehydrogenase, DPYD = dihydropyrimidine dehydrogenase gene, DPYD-AS = DPYD activity score, ECOG = Eastern Cooperative Oncology Group, EDTA = ethylenediaminetetraacetic acid, FdUMP = 5-fluoro-2'-deoxyuridine-5'-monophosphate, HFS = hand-foot syndrome, HGB = hemoglobin, INB = incremental net benefit, INR = International Normalized Ratio, mCRC = metastatic colorectal cancer, MTHFR = Methylenetetrahydrofolate reductase, MVG = Multiorgan failure due to dihydrofolate reductase and folate deficiency, pT3 = tumor size, pN1c = lymph node involvement, pM0 = no metastasis.
1. Introduction

Fluoropyrimidines such as 5-fluorouracil (5-FU) and its orally active prodrug, capecitabine are widely used in the treatment of gastrointestinal cancer including colorectal cancer (CRC). In fact, more than 2 million of patients receive these types of drugs annually. In particular, 5-FU and capecitabine are the backbone of CRC therapeutic schemes either in adjuvant than in metastatic setting. Although treatments with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX), capecitabine plus oxaliplatin (XELOX), and fluorouracil, leucovorin, and irinotecan (FOLFIRI) have improved overall survival of metastatic CRC (mCRC), 5-FU based regimes are challenging due to variability in efficacy and in toxicity among patients.\(^1\)

The mechanism of action of 5-FU has been already described and entails misincorporation of 5-FU metabolites into RNA and DNA and inhibition of thymidylate synthase (TYMS). In particular, TYMS inhibition by 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) triggers a cascade of molecular alterations that leads to misincorporation of 5-FU metabolites into DNA, impaired DNA replication, synthesis, and repair, which eventually leads to DNA breaks.\(^4\) Capecitabine is a pro-drug of the 5-FU with an oral formulation, activated to 5-FU through a 3-step enzymatic process requiring carboxylesterase (CES), cytidine deaminase (CDA) and thymidine phosphorylase (TYMP). Interestingly some tumors express high levels of TYMP, the rate-limiting enzyme activating capecitabine to 5-FU, enabling high and sustained intratumoral levels of active drug.\(^5\)

Depending on the regimen used, from 10% to 30% of patients experience side effects related to 5-FU that are classified as Grade 3 according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 such as diarrhea, nausea and vomiting, mucositis/stomatitis, myelosuppression, and hand-foot syndrome (HFS). Overall, 5-FU induces 0.5% to 1.0% mortality (grade 5) related to these side effects.\(^6,7\) Although the efficacy of capecitabine is considered to be equivalent to 5-FU, their toxicity profiles are different. Both drugs induce gastrointestinal adverse events (AEs), however, the incidence of stomatitis is significantly lower using capecitabine, whereas the incidence of diarrhea is significantly higher especially in combination with irinotecan-based regimen.\(^8,9\) Compared to intermittent 5-FU, capecitabine is associated with a lower rate of neutropenia, but HFS occurs far more frequently.\(^9\) Both drugs are known for a low prevalence of cardiovascular toxicity.\(^10\)

An important role in the genesis of 5-FU AEs is played by dihydropyrimidine dehydrogenase (DPD), which is a rate-limiting liver enzyme responsible for the degradation of more than 80% of 5-FU administered dose.\(^11\) Dihydropyrimidine dehydrogenase gene (DPYD) is a large and highly polymorphic gene with several hundreds of reported genetic variants. DPD activity levels vary considerably among individuals with consequences in 5-FU therapies for efficacy and toxicity.\(^12,13\) Single-nucleotide polymorphisms (SNPs) in DPYD may cause enzyme deficiency resulting in toxicity with 5-FU therapy. It is estimated that up to 5% of the population is deficient in DPD enzyme activity.\(^12-14\) The most well studied DPYD variant is the IVS14+1G>A, also known as DPYD *2A.\(^15\) Conversely in Europeans, the most common DPYD variant is that in HapB3, c.1129–5923C>G with carrier frequencies of 4.7%, followed by IVS14+1G>A and 2846A>T with carrier frequency of 1.6% and 0.7%, respectively. Considering the combination of all 4 variants about 7% of Europeans carries at least one decreased function DPYD variant.\(^13\) Other variants of DPYD like c.1679T>G, were studied and associated with grade 3 to 4 toxicity.\(^16\) DPD deficiency is not routinely assessed before 5-FU or capecitabine administration and its test remains an option. Several methods are available for testing DPD deficiency even if there is no recommended standardized assessment technique.

2. Material and methods

Several blood EDTA samples were collected from patient. For the analysis in our laboratory, DNA extraction, polymerase chain reaction (PCR) and hybridization were all performed using the PGX-5FU StripAssay kit (Vienna Lab) which detects only IVS14+1G>A variant of DPYD.

But more detailed analysis became necessary and so, other blood samples were sent to a more equipped laboratory. In this lab, genomic DNA was isolated from peripheral blood using the MagCore Genomic DNA Whole Blood Kit (RB Bioscience Corp. CE IVD), according to the manufacturer’s instructions. The quality of DNA samples was assessed by capillary electrophoresis and their quantity was evaluated with the Qubit 2.0 Fluorometer (Life Technologies).

Assays of samples for candidate genes polymorphisms have been performed using the “Fluoropyrimidines response” kit (Diotech Pharmacogenetics, Ancona, Italy CE IVD), according to the manufacturer’s instructions. PCR reactions were performed on the Rotor-Gene Q (Qiagen, Milan, Italy). Single-stranded DNA templates were prepared using the PyroMark Vacuum Prep Workstation (Biotage, Uppsala, Sweden). Lastly, pyrosequencing analysis was carried on the PyroMark Q96 ID (Biotage, Uppsala, Sweden). We looked for the following SNPs: MTHFR A1298C (rs1801131), MTHFR C677T, DPYD IVS14+1G/A (rs3918290), DPYD A2846T (rs6737698), DPYD T1679G (rs55886062), TSER 28bp VNTR (rs45445694).

3. Case presentation

A 46-years-old Caucasian man, with no comorbidity and familiar history of cancer after a colonoscopy made for recurrent abdominal pain had a diagnosis of adenocarcinoma poor differentiated of the left colon. A CT scan ruled out distant metastases. Thus, on July 2017 he underwent left hemicolectomy: pT3G3N1cM0. According to the disease stage, on September 2017 he should have received 8 cycles of XELOX (oxaliplatin 130mg/mq day 1, capecitabine 2000mg/day for 14 days, every 3 weeks) as adjuvant therapy. Before starting capecitabine
treatment, as our clinical practice, we performed DPYD IVS14 +1G>A mutation testing (the only available at our university) that revealed a heterozygous alteration: for this reason, the patient started the first cycle with 50% of dose reduction of capecitabine. After 5 days, he started to have diarrhea G2 and oral mucositis G3 according to CTCAE v 5.0. According to our prescription, he assumed two caplets of Loperamide after the first loose stool, 1 caplet after each subsequent loose stools and Nystatin oral suspension for the mucositis. Despite the use of supportive therapy, there was no improvement of these symptoms and on day 13 blood count revealed a leukopenia G4 (0.90 x 10^3/uL), neutropenia G4 (0.23 x 10^3/uL) and thrombocytopenia G4 (24 x 10^3/uL) according to CTCAE v 5.0. Capecitabine was immediately discontinued. For these reasons, the patient was hospitalized. He was admitted to our Oncology Unit in a wheelchair with a performance status (PS) 3 according to Eastern Cooperative Oncology Group (ECOG). On day 15, when patient was hospitalized side effects were: diarrhea G3 (more than 12 stools per day), oropharyngeal mucositis G3 with food intake completely stopped since 5 days. Blood exams revealed further decrease of white blood cells (WBC) (0.23 x 10^3/uL), of neutrophils (NEU) (0.1 x 10^3/uL), of platelets (PLT) (23 x 10^3/uL), and hyperbilirubinemia G2 (2 mg/dL) with an INR of 2.12. We immediately started an intensive rehydration therapy, antibiotic and antifungal prophylaxis (Piperacillin/Tazobactam 4.5 g x 3 times a day i.v., Clarithromycin 500 mg x 2 times a day i.v., fluconazole 400 mg daily i.v.) and Lenograstim 34 M.U.s.c. for neutropenia; Longastatin 0.1 mg/mL x 3 times a day for diarrhea and total parenteral nutrition due to mucositis. The next day the patient had been transferred to our Infections Disease Department and was moved into isolation room. During the following days, WBC count reached a stable nadir of 0.1 x 10^3/uL, from day 16 to day 24 despite daily Lenograstim administration. Platelets fell to a nadir of 3 x 10^3/uL at day 21 and he needed daily platelets transfusions (1 bag a day) from day 16 until day 22. Additional findings included a progressive liver failure with a nadir in the third week: slight INR elongation (1,5), hyperbilirubinemia G3 (6.8 mg/dL at day 23) and hypoalbuminemia (nadir 2.2 g/dL at day 22) with peripheral edemas but with no elevation of transaminases. The patient maintained his body temperature until day 22 when he had fever (37.7°C) and it was decided to add Vancomycin 125 mg capsules 4 times a day to antibiotic therapy. All the blood cultures, however, remained negative for the entire hospitalization period. Red blood cell count decreased progressively reaching G2 (HGB 9,0 g/dL) on day 19, maybe due to the diarrhea with rectal bleeding and to the delayed drug toxicity on the erythroblasts. On day 23 the patient developed a severe anemia (HGB 5.1 g/dL, G3) that required 2 bags of erythrocyte concentrate transfusion. On day 24 the clinical situation started to get better and also the blood analysis improved. On day 28 all values returned to normal (leukocytes 14.5 x 10^3/uL; NEU 10.7 x 10^3/uL; HGB 10.1 g/dL; platelets 23.3 x 10^3/uL), and also the patient fully restored his performance status. In fact, diarrhea stopped, edemas disappeared and he completely resumed oral feeding. After few days the patient was discharged from the hospital. During the last days of hospitalization, considering the patient’s extreme toxicity despite the half dose of capecitabine, three blood samples were sent to another laboratory to make further analysis. The patient was found to be compound heterozygous carrier of 2 variants: the DPYD IVS14 +1G>A and DPYD 2846A>T. Furthermore, our patient had also 2 heterozygote variants for Methylene Tetrahydrofolate Reductase (MTHFR) gene: the -C677T and the -A1298C while he had no alterations in the other 2 analyzed variants. Moreover, the patient underwent 2 whole-body CT scans, after 2 and 8 months from hospitalization, that confirmed no evidence of disease (N.E.D.). Performance status is 0 according to ECOG scale, and blood analysis are in the normal range.

4. Discussion

In this case report, our purpose is to highlight the role and clinical implication of a double heterozygote variant of DPYD gene, rarely described in the scientific literature. Only 2 other papers described 3 cases with the same variants but differently from our patient they led to the patient’s death. One explanation may be due to the fact that our patient started capecitabine treatment at 50% of the given dose.

Even though, no other variants of the DPYD gene have been evaluated beyond those mentioned, making a wide review of the literature it is probable that the reported toxicities are due only to the 2 described. These 2 alterations lead to an almost complete inhibition of the enzyme. In particular the IVS14+1G>A variant, located at the splice donor site of intron 14, leads to skipping of exon 14 during pre-mRNA splicing and consequently to a truncated protein with absent DPD activity. Instead, the 2846A>T (rs67376798, D949V) variant, located on exon 22 on the 4Fe-4S site, affects DPD activity through direct interference with cofactor binding and electron transport.[12,17-19]

In most cases of DPD deficient activity there is only 1 gene alteration and several analyses suggest that they can cause different toxicities. In fact, a metanalysis made by Terrazzino et al showed that the increased risk of overall grade ≥3 toxicity for patients carrying DPYD IVS14+1G>A and DPYD 2846A>T variants is 5- and 8-fold, respectively, compared to wild-type patients treated with fluoropyrimidines. In particular DPYD IVS14+1G>A variant is a strong risk factor of grade ≥3 hematologic toxicity and a weak risk factor of grade ≥3 mucositis and diarrhea. Instead, a strong association was also found between carriers of the DPYD 2846A>T allele and grade ≥3 diarrhea.[20] Our patient had toxicities that are in line with those reported in the literature.

In another study Deenen et al showed that the DPYD IVS14 +1G>A variant significantly is associated with the specific AEs nausea/vomiting (P = .007) and neutropenia (P < .001), whereas the DPYD 2846A>T statistically significantly associated with dehydration (P = .02), diarrhea (P = .003), leukopenia (P = .002), neutropenia (P < .001), and thrombocytopenia (P < .001).[21] Deenen et al also tried to demonstrate if these variants have an advantage in terms of overall survival associated with a greater efficacy of fluoropyrimidines, but this hypothesis was not confirmed.[21]

There is also a potential interaction of co-administered drugs modulating the influence of DPYD risk alleles on 5-FU toxicity for platinum compounds. In a small study of 22 patients, reduced DPD activity was measured after receiving treatment with platinum complexes, which suggests a partial inhibition of the DPD by these complexes.[22] In addition, our patient has also two heterozygote variants for MTHFR gene, the -C677T and the -A1298C. MTHFR is an enzyme that carries out a central reaction by irreversibly catalyzing the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate, the primary circum-


ing form of folate, which serves as a methyl-group for DNA methylation reactions. High level of 5,10-MTHF, such as in low MTHFR activity, might theoretically lead to greater inhibition of TYMS and enhanced cytotoxicity of 5-FU.\textsuperscript{[12]} Findings from 3 relatively small studies have shown an association of MTHFR –677C>T and −1298A>C with capcitabine-related AEs, whereas other six were negative.\textsuperscript{[16,24,25]} However, the recent analysis of 927 CRC patients participating in the QUASAR2 trial could not confirm the predictive value of either MTHFR –677C>T or −1298A>C for overall grade ≥ 3 toxicity, grade ≥ 3 diarrhea or grade ≥ 3 HFS.\textsuperscript{[23]}

We have found only 2 other papers in the literature that describe cases with double heterozygote variant, DPYD IVS14 +1G>A and DPYD 2846A>T, similar to our patient. In the first paper, Ezzeldin et al describe 2 patients with the same variant. The 2 patients have also alterations in other sequences of DPYD gene that do not seem to influence the enzyme’s activity.\textsuperscript{[26]} In the first patient, a 73-year-old Caucasian male with resected stage II colon cancer, was administered a regimen of 5-FU and Leucovorin at standard doses. Seven days after completing the 5-day regimen, the patient developed neutropenia, severe stomatitis, exfoliation of the skin, diarrhea, and atrial fibrillation. The patient died 16 days after. In the second patient, a 58-year-old white female with resected stage III colon cancer, was administered an adjuvant chemotherapy with Roswell Park regimen of 5-FU and Leucovorin. The patient received only day 1 of chemotherapy and after 7 days developed neutropenia, nausea, vomiting, and severe mucositis. Her condition worsened with the development of sepsis, acute respiratory distress syndrome, and hypotension. Despite aggressive therapy with systemic antibiotics and hemodynamic support, the patient died 5 weeks after hypotension. Despite aggressive therapy with systemic antibiotics development of sepsis, acute respiratory distress syndrome, and vomiting, and severe mucositis. Her condition worsened with the regimen of 5-FU and Leucovorin. The patient received only day 1 administered an adjuvant chemotherapy with Roswell Park.

The 2 patients have also alterations in other sequences of DPYD gene that do not seem to influence the enzyme’s activity.\textsuperscript{[26]} In the first patient, a 73-year-old Caucasian male with resected stage II colon cancer, was administered a regimen of 5-FU and Leucovorin at standard doses. Seven days after completing the 5-day regimen, the patient developed neutropenia, severe stomatitis, exfoliation of the skin, diarrhea, and atrial fibrillation. The patient died 16 days after. In the second patient, a 58-year-old white female with resected stage III colon cancer, was administered an adjuvant chemotherapy with Roswell Park regimen of 5-FU and Leucovorin. The patient received only day 1 of chemotherapy and after 7 days developed neutropenia, nausea, vomiting, and severe mucositis. Her condition worsened with the development of sepsis, acute respiratory distress syndrome, and hypotension. Despite aggressive therapy with systemic antibiotics and hemodynamic support, the patient died 5 weeks after receiving the single dose of 5-FU.\textsuperscript{[26]} Boisdron-Celle et al showed another similar case: this case report is interesting because it was calculated the clearance of 5-FU in the patient that resulted close to zero.\textsuperscript{[27]} In all these 3 cases, however, the chemotherapy was fatal, differentially from our patient.

One of the most important authority for the implementation of pharmacogenetics in the clinical practice is the Clinical Pharmacogenetics Implementation Consortium (CPIC) which recently did an update of their guidelines with an important variation compared with the previous ones: the addition of DPYD activity score (DPYD-AS). It is calculated as the sum of the activity scores of the 2 DPYD variants with the lowest variant activity score and includes three categories: DPYD poor metabolizers (DPYD-AS: 0 or 0,5); DPYD intermediate metabolizers (DPYD-AS: 1 or 1.5) and DPYD normal metabolizers (DPYD-AS: 2) (13). According to this classification, our patient is a poor metabolizer (DPYD-AS: 0.5), with an almost complete DPD deficiency and increased risk for severe or even fatal drug toxicity. In this setting, if no fluoropyrimidine-free regimens are considered a suitable therapeutic option, may be considered 5-FU administration at a strongly reduced dose combined with early therapeutic drug monitoring (it is estimated that a dose reduction of at least 75% would be required).

It is important to remember that not all carriers of DPYD decreased/no function variants develop severe toxicity at standard doses.\textsuperscript{[28,29]} At the same time, patients without a DPYD decreased/no function variant may still experience severe toxicity due to different genetic, environmental, or other factors.\textsuperscript{[30]}

In these circumstances, the US Food and Drug Administration and the European Medicines Agency do not currently require pharmacogenomics testing before 5-FU administration. There are also alternative or complementary tests to DPYD genotyping assessing DPD activity directly in peripheral mononuclear cells or indirectly through the endogenous dihydrouracil/uracil ratio (UH2/U) in plasma, or using a uracil loading test. These tests are no widely available before starting fluoropyrimidine-based therapy.\textsuperscript{[31]}

The benefit of upfront DPYD genotyping has been demonstrated in some recent prospective trials which probably will change the international guidelines.\textsuperscript{[30,32,33]} Boisdron-Celle et al made a prospective non-randomized study in which 2 parallel cohorts of patients treated with 5-FU-based chemotherapy were compared: in Arm A, the patients had DPD deficiency screening before treatment whereas in Arm B no pre-therapy screening was performed. The percent of patients with a toxicity grade 3 or higher observed in Arm A was 10.8% compared to 17.55% reported in Arm B ($P=.0497$). The percentage of death was reduced from 2.5/1000 in Arm B to 0 in Arm A.\textsuperscript{[32]} In another prospective trial, participants carried only the DPYD IVS14 +1G>A variant and were treated with an individualized dose of capcitabine, whereas non-carriers received full standard dose. Overall, the incidence of grade ≥ 3 toxicity was reduced from 73% in variant allele carriers receiving the standard dose to 28% by genotype-guided dosing.\textsuperscript{[30]} A similar work, recently presented at ESMO congress 2018, assessed the effect of prospective screening for four DPYD heterozygous variants (DPYD c.1905 +1G>A, c.2846A>T, c.1679T>G, and c.1236G>A in Hap B3) on patients’ safety and subsequent DPYD genotype-guided dose individualization. Results showed that this strategy has the potential to reduce toxicity risk for three of the 4 analyzed variants: the RR for severe fluoropyrimidine-related toxicity was 1.31 for genotype guided dosing compared with 2.87 in the historical cohort for DPYD c.1905+1G>A carriers, no toxicity compared with 4.30 in c.1679T>G carriers, 2.00 compared with 3.11 for c.2846A>T carriers, and 1.69 compared with 1.72 for c.1236G>A carriers. No toxicity-related deaths were observed in carriers of DPYD alterations after genotype-guided dose reduction (except for one due to protocol violation).\textsuperscript{[33]} All these studies also demonstrated that this strategy is also cost saving. An interesting medico-economic study by Traoré et al showed that the cost of a pre-treatment screening test combining genetic and phenotyping was €190. The avoided cost per patient screened was €313 for 2 cycles of treatment and a savings of €2780 per toxicity avoided. The incremental net benefit (INB) per patient screened was €426. The screening strategy allowed for avoiding toxicities and saving money.\textsuperscript{[34]}

In conclusion, on our opinion, even if there is not a great availability yet, implementation of pre-treatment genotypic tests to individualize fluorouracil (5-FU)-based therapy by national regulatory agencies, allows a safer and individualized approach to chemotherapy management.

Author contributions
Conceptualization: Vincenzo De Falco, Erika Martinelli.
Data curation: Vincenzo De Falco, Stefania Napolitano, Giovanni Conzo, Erika Martinelli, Nicoletta Zanaletti, Pasquale Vitale, Emilio Francesco Giunta, Pietro Paolo Vitelli, Teresa Troiani.
Investigation: Vincenzo De Falco, Maria Iole Naticchichio, Nicola Coppola, Giovanni Conzo, Nicoletta Zanaletti, Pasquale Vitale, Emilio Francesco Giunta, Maria Teresa Vietri, Davide Ciardiello, Anna Marinaccio, Teresa Troiani.
Methodology: Stefania Napolitano, Nicola Coppola, Maria Teresa Vietri.

Supervision: Ferdinando De Vita, Fortunato Ciardiello, Teresa Troiani.

Validation: Maria Iole Natalicchio, Nicola Coppola, Erika Martinelli, Pietro Paolo Vitiello, Ferdinando De Vita, Fortunato Ciardiello, Teresa Troiani.

Visualization: Stefania Napolitano, Pietro Paolo Vitiello, Ferdinando De Vita, Fortunato Ciardiello, Teresa Troiani.

Writing – original draft: Vincenzo De Falco.

Writing – review & editing: Fortunato Ciardiello, Teresa Troiani.

References

[1] de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000;18:2938–47.

[2] Cassidy J, Tabernero J, Twelves C, et al. XELOX (capecitabine plus oxaliplatin): active first-line therapy for patients with metastatic colorectal cancer. J Clin Oncol 2004;22:2084–91.

[3] Douillard JY, Cunningham D, Roth AD, et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. J Clin Cancer Res 2000;6:4705–12.

[4] van Kuilenburg AB, Haasnoot J, Richel DJ, et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. J Clin Cancer Res 2000;6:4705–12.

[5] Bonotto M, Bozza C, Di CL, et al. Making capecitabine targeted therapy effective and a double-dose regimen for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000;355:1041–7.

[6] Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003;3:330–8.

[7] Twelves C, Wong A, Nowacki MP, et al. Capecitabine as adjuvant treatment for stage III colon cancer. N Engl J Med 2005;352:2696–7.

[8] Henricks LM, Lunenburg CATC, de Man FM, et al. DPYD genotype- guided dose individualisation of 5-fluorouracil and 5-fluorouracil-related toxicity: ready for prime time. Eur J Cancer 2016;54:40–8.

[9] Boisdron-Celle M, Remaud G, Traore S, et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. Cancer Lett 2007;249:271–82.

[10] Meulendijks D, Henricks LM, Sonke GS, et al. Clinical relevance of DPYD variants c.1679T>G, c.1236G>A and c.1601G>A as predictors of severe 5-fluorouracil-associated toxicity: a systematic review and meta-analysis of individual patient data. Lancet Oncol 2015;16:1639–50.

[11] Morel A, Boisdron-Celle M, Fey L, et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. Mol Cancer Ther 2006;5:2893–904.

[12] Deenen MJ, Meulendijks D, Cats A, et al. Upfront genotyping of DPYD*2A to individualize fluoropyrimidine therapy: a safety and cost analysis. J Clin Oncol 2016;34:227–34.

[13] Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol 2016;27:1386–422.

[14] Boisdron-Celle M, Captain O, Faroux R, et al. Prevention of 5-fluorouracil-induced early severe toxicity by pre-therapeutic dihydropyrimidine dehydrogenase deficiency screening: assessment of a multiparametric approach. Semin Oncol 2017;44:13–23.

[15] Henricks LM, Lunenburg CATC, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Lancet Oncol 2018.

[16] Traoré S, Boisdron-Celle M, Hunault C, et al. DPD deficiency: medicoeconomic evaluation of pretreatment screening of 5-FU toxicity. JCO 2012;30(4_suppl):410.