# Sudden Death Related to Toxicity in a Patient on Capecitabine and Irinotecan Plus Bevacizumab Intake: Pharmacogenetic Implications

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## Introduction

Capecitabine is an oral alternative to fluorouracil (FU) frequently administered as part of combination therapies in digestive oncology. This prodrug is designed to be activated through a triple enzymatic process, which eventually generates FU in tumors. A genetic polymorphism that affects dihydropyrimidine dehydrogenase (*DPYD*), which is the enzyme responsible for the liver detoxification of FU, is the canonical syndrome identified as a possible pharmacogenetic issue with capecitabine.[1,2] However, an early step in the hepatic activation of capecitabine requires cytidine deaminase (CDA), which is a ubiquitous enzyme also affected by several genetic polymorphisms.[3,4] In digestive oncology, high interpatient variability observed in CDA activity is a rising concern with gemcitabine that is detoxified in the liver by deamination. Downregulated CDA has been associated with overexposure and subsequent severe toxicities on gemcitabine treatment.[5-8] Because CDA also plays a critical role in the activation of capecitabine to FU, the reported variability in its activity could markedly affect FU formation, with either a loss of efficacy (CDA-deficient patients) or increased toxicities (CDA-ultrametabolizer patients). Little data are available about the impact of CDA status on the clinical outcome with capecitabine treatment. We previously published the case of a patient with increased CDA activity who experienced severe toxicities after capecitabine administration.[9] More recently, it was reported that the deleted allele rs3215400 across the *CDA* promoter could be predictive of severe hand-foot syndrome in patients treated with capecitabine,[10] although a previous report failed to evidence this association,[11] which thus illustrated the conflictual genotype-to-phenotype relationships with CDA.[12]

## Case Report

This patient case was that of a 57-year-old white man treated for metastatic colorectal cancer. On first screen, anemia was found, and endoscopy with biopsy revealed a well-differentiated adenocarcinoma. The patient was initially treated by surgery in 2007 with colectomy and hepatic metastasectomy. Because dihydropyrimidine dehydrogenase (DPD) deficiency is a condition associated with increased risk of life-threatening toxicities in patients scheduled for an FU-based regimen, DPD status was evaluated according to the standard uracil-to-dihydrouracil plasma ratio determination, as described previously.[1] After preliminary assessment of the functional status of DPD showed no evidence of deficiency (ie, uracil-to-dihydrouracil ratio $< 2$), standard adjuvant chemotherapy with infusional FU, leucovorin, and oxaliplatin was initiated. Treatment was well tolerated. However, at the end of the adjuvant therapy, new lung and liver metastases were observed, and the patient was treated with infusional FU, leucovorin, and irinotecan in combination with bevacizumab. This

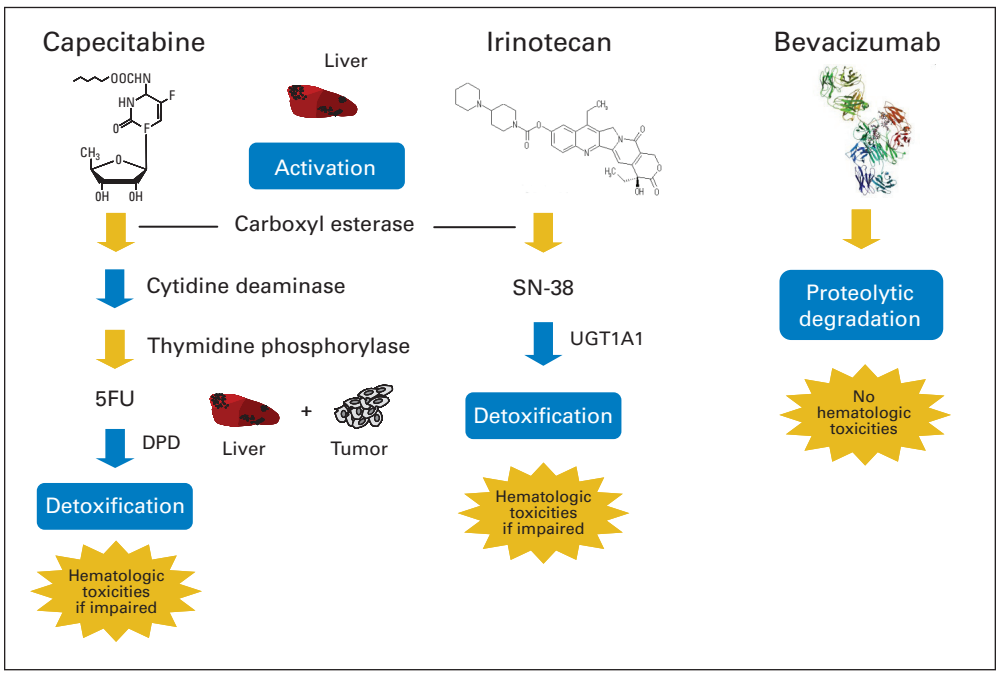


**Fig 1.**

**Table 1.** Genotypic and Phenotypic Investigations on the DPD, UGT1A1, TYMS, and CDA Enzymes

| Gene Symbol | Polymorphism ID | Function | Patient Genotype | Patient Phenotype |
|---|---|---|---|---|
| *DPYD* | c.464 T>A (exon 5) | Stop codon | T/T | Normal (U:UH2 < 2) |
| | c.1679 T>G (exon 13) | Missense | T/T | |
| | IVS14 + 1 G>A (exon 14), rs3918290 | Exon 14 skipping | G/G | |
| | c.2846 A>T (exon 22) | Missense | A/A | |
| *UGT1A1* | UGT1A1*28 $(TA)_6$>$(TA)_7$, rs8175347 | 5′UTR | $(TA)_6/(TA)_6$ | Not assessed |
| *TYMS* | 28-bp tandem repeat, rs34743033 | 5′UTR | 3R/3R | Not assessed |
| | c.-58G>C, rs2853542 | 5′UTR | G/C | |
| | 1494del TTAAAG, rs34489327 | 3′UTR | 0pb/6pb | |
| *CDA* | −31delC, rs3215400 | 5′UTR | -/C | Ultrametabolizer (CDA, 9.1 U/mg) |
| | c.79 A>C (exon 1), rs2072671 | Missense | A/C | |
| | c.208G>A (exon 2), rs60369023 | Missense | G/G | |
| | c.435C>T (exon 4), rs1048977 | Synonymous | C/T | |

Abbreviations: bp, base pair; CDA, cytidine deaminase; DPD, dihydropyrimidine dehydrogenase; ID, identifier; TYMS, thymidylate synthase; U:UH2, uracil-to-dihydrouracil ratio; UGT1A1, uridine glucuronyl transferase 1A1; UTR, untranslated region.

combination was also well tolerated with no signs of systemic toxicity. However, after 32 chemotherapy cycles over a span of 2 years, the central catheter was removed as a result of cutaneous intolerance. The patient was to be treated with a protocol of capecitabine plus irinotecan and bevacizumab as follows: irinotecan 200 mg/m$^2$ (total dose, 400 mg) on days 1 and 21, capecitabine 1,000 mg/m$^2$ twice daily from days 1 to 14 (total dose, 3500 mg/d), and bevacizumab 7.5 mg/kg (total dose, 775 mg) on days 1 and 21. However, soon after the treatment began (day 7), the patient was rehospitalized as a result of severe toxicities (eg, grade 4 diarrhea, grade 4 neutropenia, and sepsis). Capecitabine intake was immediately discontinued. Despite the appropriate symptomatic treatment (clavulanic acid, amikacin, and filgrastim), the condition of the patient quickly deteriorated with a fatal outcome on day 12.

## Discussion

Because genetic polymorphisms that affect the disposition of anticancer agents are now a major issue in clinical oncology,[13] we investigated whether dysregulation of the various enzymes involved in the activation/deactivation patterns of the administered drugs could have been responsible for the death of the patient (Fig 1; SN-38, toxic metabolite 7-ethyl-10-hydroxy-camptothecin). The investigations assessed both the functional and genetic status of the patient, with the results listed in Table 1. Because this patient had tolerated extensive irinotecan exposure over 2 years, it was believed to be unlikely that the inherited pharmacogenetic syndromes that affect *UGT1A1* (eg, *UGT1A1*28* allelic variant) usually associated with severe toxicities with irinotecan treatment could have explained this fatal outcome.[14] Postmortem genetic investigations confirmed that this patient was bearing the *UGT1A1*1* common genotype ($[TA]_6/[TA]_6$) and not the allelic variant. Bevacizumab was also eliminated as the cause for this toxicity on the basis of the evidence that the observed toxicities in this patient were inconsistent with those previously described for this drug[15] and the knowledge that the patient had tolerated extensive previous treatment with bevacizumab. Therefore, the focus of the investigation turned to capecitabine. The mechanism by which capecitabine may cause serious toxicities may be related to increased FU exposure that results from either impaired detoxification by DPD or increased formation of FU from capecitabine. DPD is the rate-limiting enzyme for detoxification of fluoropyrimidine drugs, and genetic polymorphism that affects *DPYD* is a paradigmatic pharmacogenetic syndrome associated with early severe toxicities with FU derivatives.[16,17] As described previously, the patient had been identified as non–DPD deficient, which was a finding that was consistent with his tolerance of a previous regimen of infusional fluorouracil, leucovorin, and oxaliplatin and then fluorouracil, leucovorin, and irinotecan. To further confirm this nondeficient phenotype, a retrospective assessment of *DPYD* genetic status showed none of the genetic variations (ie, exon 5 464 T>A, exon 13 1679 T>G, exon 14 IVS14 + 1 G>A, and exon 22 2846 A>T) usually associated with DPD impairment.[18] Thus, an inherited inability to detoxify circulating FU was not the cause of the death related to toxicity. Additionally, we investigated polymorphisms in the 5′ and 3′ untranslated regions of the *TYMS* gene that are responsible for the dysregulation of thymidylate synthase and were previously associated with increased toxicities in patients treated with capecitabine.[19] Similar to *DPYD*, no functionally relevant polymorphisms were found on *TYMS*, and the genotype was typically associated with normal thymidylate synthase expression,[20,21] which was an

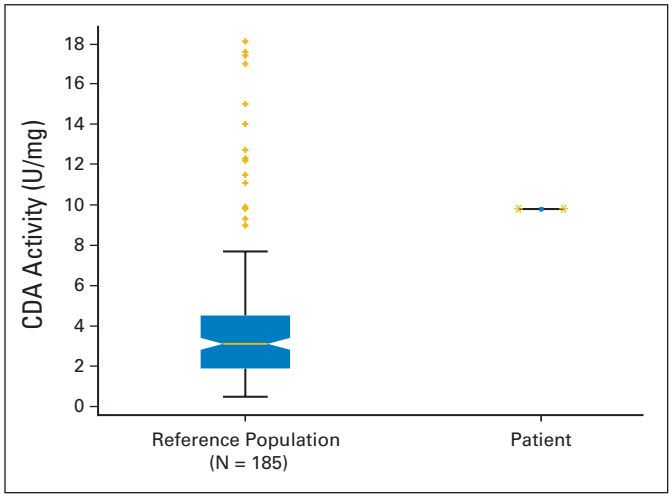


**Fig 2.**

  

observation that implicated increased hepatic conversion of the prodrug capecitabine to FU as the last remaining cause of the lethal toxicities. As a triple prodrug, capecitabine is rationally designed to be preferentially activated to FU by carboxylesterase, CDA, and finally thymidine phosphorylase.[22] Because our group had previously investigated *CDA* dysregulations as a means to identify deficient individuals likely to develop severe toxicities with gemcitabine,[6,8] we have already observed that some patients displayed particularly increased CDA activities and, thus, could be considered CDA ultrarapid metabolizers (UMs). Thus, we hypothesized that the patient described in this article could have been a UM, which resulted in increased formation of FU and subsequent lethal toxicities. To test this hypothesis, a phenotypic investigation was first carried out to establish the functional CDA status of this patient. An evaluation of CDA residual activity in serum was performed as a surrogate for CDA status.[8] The CDA activity of the patient was 9.1 U/mg, which was 193% higher than the median value recorded in patients with cancer at our institute (mean, 3.9 $\pm$ 3.3 U/mg; n = 185). Regarding the general distribution in CDA activities normally observed (Fig 2), this patient was unequivocally classified as a UM because his CDA value differed statistically from the reference population ($t_{obs} > t_{student}$; $\alpha = 0.01$; n = 184), which confirmed our hypothesis. Additional genetic investigations showed several polymorphisms on the *CDA* gene. Heterozygocity was found on the 435CT and 79AC mutations. However, conflicting reports of the genotype-to-phenotype relationships of these polymorphisms prevented making a conclusion.[12] More interestingly, the patient was also heterozygous for the rs3215400 single nucleotide polymorphism that corresponds to a C insertion at the −31 position. Caronia et al[10] have recently shown that this insertion was associated with enhanced CDA expression, probably through the creation of an additional binding site for the transcriptional factor E2F, with a possible impact on increased activity. Overall, both our functional and genetic data strongly suggested, for the first time to our knowledge, that deregulated *CDA* with subsequent extensive activity could be the initiating factor for death related to toxicity in a capecitabine-treated patient, which is likely produced by higher amounts of circulating FU than normally expected from the standard dosage. Although this patient did not have an inherited deficiency in DPD, the role of DPD as a rate-limiting, saturable downstream enzyme in the elimination of fluoropyrimidines may have prevented this patient from detoxifying the unexpectedly high levels of FU generated from capecitabine, which led to unrecoverable toxicities and death eventually.

Improving the use of anticancer drugs through personalized medicine is of great interest in clinical oncology. Deregulations and genetic polymorphisms that affect *TPMT*, *UGT1A1*, *DPYD*, *Cyp2D6*, and *CDA* are regularly associated with highly variable pharmacokinetics and poor clinical outcomes with mercaptopurine, irinotecan, FU plus capecitabine, tamoxifen, and gemcitabine, respectively.[23,24] We previously reported the first case of death related to toxicity, to our knowledge, in a patient who was undergoing a capecitabine plus oxaliplatin protocol related to a *DPYD* 1896TC polymorphism with subsequent profound DPD deficiency.[1] Consequently, the *DPYD* genetic polymorphism is now fully recognized as a major risk for the development of severe/lethal toxicities with capecitabine, and known DPD deficiency is a contraindication with this drug. Because capecitabine is usually a well-tolerated oral drug, it is often seen as a safe and convenient alternative in patients who experienced FU-related toxicities. However, in this article, we demonstrated that patients without DPD deficiency, who, furthermore, proved to fully tolerate FU in the past, are at risk for FU lethal toxicities as a result of CDA UM status if administered oral capecitabine. Although additional clinical investigations are required to fully confirm the implication of CDA in unexpected, severe toxicities with this drug, this report suggests that, beside the genetic polymorphisms affecting *DPYD*, deregulations of *CDA* should be screened at bedside in patients scheduled for capecitabine-based therapy.

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