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Capecitabine pharmacogenetics: historical milestones and progress toward clinical implementation

“...in the past decade substantial progress has been made in the recognition and understanding of the genetic underpinnings of variability in capecitabine-related toxicity, which has led to genotypic considerations being incorporated into dosing recommendations.”

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The third-generation, orally-available fluoropyrimidine capecitabine (Xeloda™, Hoffmann-La Roche, Basel, Switzerland) has approved indications for the front-line therapy of advanced and/or metastatic colorectal cancer and pretreated metastatic breast cancer. Being a 5-fluorouracil prodrug, it has also increasingly substituted infusional fluorouracil as the fluoropyrimidine backbone in chemotherapy regimens for advanced esophago-gastric malignancies on the basis of equivalent efficacy and its convenient outpatient oral dosing schedule [1–3]. Despite the proven efficacy and tolerability profile of capecitabine, selection of an optimal starting dosage remains a challenge, and clinical practices for treatment initiation differ worldwide [4]. Stark interindividual and inter-regional heterogeneity exists with respect to toxicity and efficacy profiles, and may be partially explained by genetic variation, with the most well-established pharmacogenes to-date being *DPYD* and *TYMS*. In this *Editorial*, we highlight several historical milestones and recent progress that have added to the evidence base for clinical implementation of pharmacogenetics-guided capecitabine dosing.

To better appreciate the potential role of pharmacogenetics in personalized capecitabine chemotherapy, it seems apposite to take stock of its clinical development and postmarketing pharmacovigilance. The US FDA-approved marketing dosage of 1250 mg/m² twice a day every 2 weeks

on/1 week off (intermittent schedule) arguably has roots in an initial Phase I trial, which dose-escalated patients with a variety of solid tumors to capecitabine monotherapy based on the intermittent schedule [5], as well as a randomized Phase II study in advanced colorectal cancer where this schedule proved superior to two alternative dosing schedules in terms of time-to-progression (TTP; median TTP: 230 vs 127 and 165 days) and dose intensity [6]. However, early anecdotal accounts from US oncologists suggested that dose reductions may improve the tolerability of capecitabine without compromising its efficacy [7]. In 2005, a post-marketing retrospective review conducted at the MD Anderson Cancer Centre, which included 113 evaluable consecutive women with metastatic breast cancer, the majority (76%) of whom were Caucasian [7], argued that a lower dosage of 1000 mg/m² may be associated with a more favorable therapeutic index, as evidenced by reduced incidence of grade 3/4 toxic effects including hand–foot syndrome (20 vs 33%), diarrhea (3 vs 13%) and stomatitis (3 vs 8%), and a lack of appreciable difference in response rates or TTP duration [7].

The improved therapeutic index of lower-dosage capecitabine among patients of Caucasian descent was supported in 2008 by a multinational, retrospective analysis of three large randomized controlled trials by Haller *et al.* in the *Journal of Clinical Oncol-*

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ogy [4], which was one of the first major reports to highlight the pharmacoethnicity of fluoropyrimidine agents. The analysis included a total of 3053 colorectal cancer patients from the USA, Europe and Asia who received capecitabine with or without oxaliplatin, or bolus 5-fluorouracil/leucovorin, and was adjusted for potential confounding factors (age, gender, treatment regimen and creatinine clearance). Confirming the anecdotal accounts, US patients were at significantly elevated risk for grade 3/4 gastrointestinal adverse events (risk ratio: 3.62; 95% CI: 2.11–6.20; $p < 0.001$) and drug discontinuation (risk ratio: 1.84; 95% CI: 1.14–2.96; $p < 0.001$) compared with patients from east Asia [4]. Furthermore, market data indicated that US physicians routinely initiate capecitabine at a lower dosage of 1000 mg/m² on the intermittent schedule, further corroborating the notion that a lower dosage empirically produces a more favorable therapeutic index among Caucasian patients [4,7].

“Compared with the hypothesis-driven candidate gene approach, which has been the preferred methodology in previous pharmacogenetic analyses, the agnostic genome-wide association study strategy would be tremendously helpful for elucidating the complete pharmacogenetic repertoire of capecitabine-related toxicities.”

At the time, evidence that germinal polymorphisms in the metabolic and pharmacodynamic pathways of fluoropyrimidine drugs may modulate fluoropyrimidine toxicity risk was also emerging [4,8,9]. Schwab *et al.* reported in 2008 that several genetic polymorphisms, especially those in *DPYD*, were highly predictive of severe fluorouracil toxicities [9]. Indeed, one of the best established genetic causes of fluoropyrimidine intolerance is deficiency of DPD, the rate-limiting enzyme involved in the catabolism of 5-fluorouracil that is encoded by the *DPYD* gene. Recent large meta-analyses have confirmed that the presence of several deleterious *DPYD* variants, including *2A, c.2846A>T, c.1679T>G and c.1236G>A/HapB3 is associated with severe toxic reactions to fluoropyrimidine drugs ([10–12] and references contained within). Both the Clinical Pharmacogenetics Implementation Consortium and Royal Dutch Association for the Advancement of Pharmacy–Pharmacogenetics Working Group have since embraced fluoropyrimidine dosing guidelines based on *DPYD* genotypes [13,14], and a recent Perspective in this journal espoused the possibility of using a *DPYD* gene activity score to tailor fluoropyrimidine dosages [15]. However, many of these *DPYD* variants are rare with a heterozygote frequency of about 1% or less among white people [10], which provides relatively

low sensitivity to identify patients at risk of severe toxicity. For instance, the combined sensitivity of genotyping c.1905+1G>A, c.1601G>A, c.1679T>G and c.2846A>T for grade 3/4 fluoropyrimidine-related toxicity is 23% (despite having a positive predictive value of >99% and negative predictive value of 80%) [16], underscoring the need to identify additional markers of fluoropyrimidine-related toxicity.

However, pharmacogenetic analyses were also focused on the major drug target of capecitabine, thymidylate synthase (TS), which is encoded by the *TYMS* gene. TS inhibition by the active metabolite fluorodeoxyuridine monophosphate prevents the formation of thymidylate (dTTP), which is a precursor for DNA synthesis, leading to cell-cycle arrest and apoptosis. Unlike *DPYD*, the *TYMS* gene harbors common functional variation, and the prominent interethnic variability in allelic frequencies mirrors inter-regional differences in capecitabine toxicity rates [4,8]. The *TYMS* promoter enhancer region (*TSE*R) contains a variable number of 28-bp tandem repeats polymorphism, which usually occur in duplex (2R) and triplet (3R) forms, with the latter being more transcriptionally active. The 3R allele is predominant in east Asian populations and the 3R/3R genotype is approximately twice as common among the Chinese population (67%) compared with the Caucasian population (38%) [17]. Retrospective analyses have reported grade 3/4 toxicity rates of 43–63%, 18–32% and 3–27% in patients carrying the 2R/2R, 2R/3R and 3R/3R genotypes, respectively, who received fluorouracil-based chemotherapy [9,18], and a recent meta-analysis (five studies including 1303 patients) demonstrated that each additional copy of the 2R allele conveyed a 36% (95% CI: 15–60%; $p < 0.001$, fixed-effects model) increased odds of experiencing grade 3 or higher global capecitabine toxicity [12]. On the other, the 3R/3R genotype appears to be associated with fluoropyrimidine resistance [18,19], warranting the question whether patients with this genotype might stand to benefit from higher dose capecitabine.

As interethnic differences in the allelic frequencies of common *TSE*R variants seemed to provide a compelling basis for the substantial inter-regional disparities in capecitabine toxicity and efficacy profiles observed [4,8,9], we asked if it may be possible to tailor capecitabine dosages according to *TSE*R genotype [18]. We, therefore, performed a *TSE*R genotype-guided Phase I dose-escalation study to determine the maximum tolerable dose and recommended Phase II dose (RP2D) for patients with the *TSE*R 3R/3R or 2R/_ genotypes [18]. The classification of 2R/2R and 2R/3R into a single arm (2R/_) was planned *a priori* because the prevalence of these genotypes are uncommon in the

Asian setting from which patients were recruited, and accrual was expected to be challenging. Capecitabine was dose-escalated in patients with advanced and/or metastatic solid tumors from 1250 to 1625 mg/m² twice a day in intervals of 125 mg/m² on the intermittent schedule, separately in TSER 3R/3R (n = 18) or 2R/2R+2R/3R (n = 5) patients. With the stratification of patients by genotypes, the maximum tolerable dose and RP2D were declared at the 1625 and 1500 mg/m² dose levels for 3R/3R patients, which are considerably higher than the empirical dosage of 1000 mg/m² that is frequently prescribed by US practitioners [4,7,18]. However, dosage recommendations for patients with the TSER 2R/_ genotypes could not be determined due to poor accrual. It is worth noting that whereas most dose modifications in the oncological sphere typically entail a dose reduction, the study proposes that a subset of patients with the 3R/3R genotype may in fact be amenable to a dose increment. Nonetheless, the clinical activity of a higher capecitabine dosage compared with the approved dosage for TSER 3R/3R patients remains to be evaluated in a Phase II study.

In summary, in the past decade substantial progress has been made in the recognition and understanding of the genetic underpinnings of variability in capecitabine-related toxicity, which has led to genotypic considerations being incorporated into dosing recommendations [13–15,18]. However, cautious optimism with respect to the clinical utility of pre-emptive pharmacogenetic testing is warranted: an SNP panel comprising well-established *DPYD* and *TYMS* variants only provides 26% sensitivity, 86% specificity and 49% positive predictive value, which would be suboptimal to inform routine clinical decision-making [12]. Evidently, much work remains to identify and validate additional markers of capecitabine toxicity. Compared with the hypothesis-driven candidate gene approach, which has been the preferred methodology in previous pharmacogenetic analy-

ses [9–12,16], the agnostic genome-wide association study strategy would be tremendously helpful for elucidating the complete pharmacogenetic repertoire of capecitabine-related toxicities. A prospective genome-wide association study of breast cancer patients treated with single-agent capecitabine from Singapore will soon be reported [20] and will reveal the extent to which common and rare variation modulates the risk of hand–foot syndrome, a common disfiguring dermatologic toxic reaction to capecitabine. Another area for consideration in future and ongoing studies is to prioritize patients who received capecitabine monotherapy (as opposed to capecitabine-containing combination regimens or who received other fluoropyrimidine drugs), as this would allow more accurate delineation of the pharmacogenetic associations with capecitabine toxicity. In fact, the clinical relevance of pharmacogenetics in capecitabine-containing regimens, where the dosage of capecitabine and the risk of acute side effects is lower, should be investigated. Finally, in selecting an appropriate dosage of capecitabine for an individual, it is important to also consider nongenetic factors such as dietary folate intake, as well as clinicopathological characteristics such as hepatorenal function, gender and age [1,8,9]. Resolution of these issues will be crucial to unlock the full potential of pharmacogenetics in personalized capecitabine chemotherapy.

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