Letter to the Editor

Correspondence re: Raida, M. *et al.*, Prevalence of a Common Point Mutation in the Dihydropyrimidine Dehydrogenase (DPD) Gene within the 5′-Splice Donor Site of Intron 14 in Patients with Severe 5-Fluorouracil (5-FU)-related Toxicity Compared with Controls. Clin. Cancer Res., *7:* 2832–2839, 2001.

Raida *et al.* (1), in the September 2001 issue of Clinical Cancer Research, investigated the genetic basis of dihydropyrimidine dehydrogenase deficiency in cancer patients who developed grade 3–4 toxicity after 5-FU[2]-containing regimens. Twenty-five patients were genotyped for the exon 14-skipping G→A mutation in the *DPYD* gene (*DPYD***2A*). This mutation was found in 6 of 25 cancer patients with severe toxicities. The high frequency (24%) of *DPYD***2A* allele in this selected group of patients compared with normal individuals led the authors to the conclusion that patients should be screened for *DPYD***2A* allele before undergoing 5-FU.

The findings of Raida *et al.* (1) increase our knowledge regarding the molecular basis of dihydropyrimidine dehydrogenase genetic deficiency, but the identification of patients at risk for severe toxicity is still an unattained goal. We believe that the use of *DPYD***2A* genotyping for routine screening is not indicated.

The clinical use of a diagnostic test derives from its sensitivity (percentage of true positives) and specificity (percentage of true negatives; Refs. 2, 3). Raida *et al.* (1) demonstrate that *DPYD***2A* test has low sensitivity and unknown specificity for predicting toxicity. The sensitivity is ~24%, with a 95% confidence interval of 7–41% (3). Concerning the specificity of the test, the percentage of positive patients that will eventually develop severe toxicity has never been estimated, making the specificity unknown. Even assuming 100% specificity (all of the positive patients will develop severe toxicity after 5-FU), this test will not be able to enlarge the therapeutic window of 5-FU because of its low sensitivity.

Frequency of *DPYD***2A* heterozygotes is 1.8% in Caucasians (4). The "best case" scenario based on the available data would be a specificity of 100% and a sensitivity of 41%. All of the patients with at least one *DPYD***2A* allele (~1.8%) would be at risk for severe toxicity, and this would represent 41% of all such individuals (1.8%/0.41 or 4.4%). In this scenario, the reduction in the percentage of patients with severe toxicity would be only 1.8%, leaving 2.6% of patients who would not be detected before treatment.

Clinical application of genotyping procedures as a tool for individualized therapy has to be validated and benefit estimated. Misinterpretation and misapplication of pharmacogenetic findings should alert investigators in the field that recommended procedures for diagnostic use of genetic tests should be outlined and decision analysis applied. Tests for detection of *DPYD* mutations are still of investigational use and should not be used outside of clinical trials.

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[2] The abbreviations used are: 5-FU, 5-fluorouracil.

Reply

The letter by Innocenti and Ratain (1) suffers from confusing remarks on statistical measures of diagnostic validity and predictive values.

The reverse transcription-PCR-based assay for the identification of the exon 14-skipping (DPYD*2A) G→A mutation in the DPYD gene detects this particular mutation. Therefore, its utility as a diagnostic test has to primarily be considered as the ability to detect this mutation. In this respect the assay has a sensitivity of 100%. In addition the specificity, the probability of a negative assay result in patients with no point mutation is 100%.

In regard to the clinical utility of the test to predict 5-FU[1] toxicity, it needs to be pointed out that there are numerous causes for the occurrence of 5-FU toxicity, among them mutations in the DPYD gene. Given this situation, it is not possible to consider sensitivity and specificity of the DPYD*2A mutation assay in a way as if there was a monocausal relationship between the DPYD*2A mutation 5-FU toxicity in general.

Our paper (2) showed that the prevalence of this point mutation in a cohort of 851 control persons was 0.94%. In contrast, among 25 patients suffering from WHO grade 3–4 toxicity a prevalence of the DPYD*2A mutation of 24% was detected. These figures are also supported by independent studies (3). In other words, the estimated probability Pr(Mut Tox) of observing an exon 14-skipping mutation given an observed severe 5-FU-related toxicity was 24%, with an exact 95% confidence interval of 11–43% (4).

However, this way of considering our data is, for the above mentioned reasons, of no primary interest in quantifying the clinical utility of screening for the DPYD*2A mutation. Rather, the clinical utility has to be determined by the probability Pr(Tox Mut) of observing a grade 3–4 toxicity given that an DPYD*2A mutation was detected.

Applying Bayes' theorem (5), this probability can be calculated as follows: $Pr(\text{Tox Mut}) = Pr(\text{Mut Tox}) \times Pr(\text{Tox}) / [Pr(\text{Mut Tox}) \times Pr(\text{Tox}) + Pr(\text{Mut no Tox}) \times Pr(\text{no Tox})]$.

Assuming the overall probability Pr(Tox) of observing any severe 5-FU related toxicity is ~4%, the probability of any severe 5-FU related toxicity given a mutation was detected can be calculated as 51.5%. This is already a worst case scenario, because here we assumed that the probability of the mutation among patients with no toxicity is 0.94%, *i.e.*, identical to the prevalence. However, a recent prospective study (6) has detected among seven prospectively identified carriers of the DPYD*2A mutation three patients who suffered WHO grade 3–4 toxicity and two patients with WHO grade 2 toxicity. Only two carriers of the mutation did not experience toxicity during their 5-FU therapy.

In addition, the same procedure can be applied for quantifying the reduction of severe 5-FU related-toxicity if all of the patients with mutations were excluded from 5-FU therapy. On the basis of the assumptions above the resulting frequency of toxicity would be 3.1% compared with 4% without any screening for mutations. Thus, the overall frequency of toxic side effects can be reduced by 22.5%. If one considers the multiplicity of causes for 5-FU toxicity, this is a remarkably powerful reduction in toxicity by screening for just this one DPYD*2A mutation.

Currently we have collected toxicity data for 31 patients who were retrospectively identified as carriers of DPYD*2A because of severe 5-FU toxicity.[2] All of these patients required very expensive medical care (growth factors, parenteral nutrition, broad spectrum antibiotics, platelet replacements, and so forth). Nonetheless, 8 of these 31 patients died from their treatment-related toxicities; 3 of them had received 5-FU in an adjuvant setting. Toxicities occurred with high- and low-dose 5-FU, with bolus and infusion regimens, as well as with Xeloda. The costs per treatment ranged from a minimum of $30,000 to much more than $100,000.

To illustrate the clinical dimension it may be recalled that in the United States alone approximately 250,000–300,000 patients are treated annually with a chemotherapeutic scheme that contains 5-FU. Given the prevalence of 0.94% of the DPYD*2A mutation approximately 2350–2820 patients carry the DPYD*2A mutation and assuming the estimated (worst case) probability of 51.5% 1210–1452 patients will suffer from severe 5-FU toxicity, and some of them will die from their treatment. This creates annual treatment cost ranging between $36.3 million and $145 million. In contrast costs for screening ($100/test) for all patients amount to $25 and 30 million, which results in savings of $11.3–115 million.

Therefore, we maintain that the currently available data provide a solid basis to call for a general pretherapeutic screening for the DPYD*2A mutation to reduce toxic side effects of 5-FU chemotherapy. Considerations of testing patients only within clinical studies would not only put a significant number of patients annually at risk to unnecessarily suffer severe toxic side effects of their therapy even to the extent that they may die but also continues to cause unnecessarily high treatment costs.

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[1] The abbreviation used is: 5-FU, 5-fluorouracil.

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