

# ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

Section Editor: Mark J. Ratain, MD

## Current Insights Into the Impact of Dihydropyrimidine Dehydrogenase Deficiency in Patients Receiving Treatment With 5-Fluorouracil



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### H&O Can genetic factors impact the efficacy and toxicity of anticancer agents?

**RD** Genes encode for various enzymes that are important in drug metabolism. As we learn more about the human genome and the genes that are responsible for these enzymes, it has become clear that defects in these genes can impact the efficacy and toxicity of chemotherapy.

There are several types of defects. There may be various mutations within the coding region of the gene, that is, the exons. Defects can also occur in the immediate area between introns (ie, the spaces between the exons) and exons, which can lead to exon skipping. Lastly, there can also be defects in the promoter region and in the introns. Genetic defects can alter the amount of transcription, which can ultimately impact the level of enzyme critical for drug metabolism, thereby changing a drug's efficacy and adverse events.

### H&O What prompted your interest in pharmacogenetics?

**RD** Like many situations in medicine, my own interest in pharmacogenetics arose from a patient. Almost 30 years ago, I had the opportunity to see a breast cancer patient who developed severe toxicity, including cytopenias, after

receiving 5-fluorouracil (5-FU) as part of a cyclophosphamide/methotrexate/5-FU (CMF) regimen. Despite attempts on the second and third cycles of administration to deliver reduced amounts of 5-FU to minimize observed toxicity, severe toxicity reoccurred, and the patient eventually became comatose. It was a very striking reaction to 5-FU, unlike what is usually seen with this typically well-tolerated drug.

We performed several studies at the time in this patient trying to understand what was happening. We determined that the patient's neurologic status was not attributable to brain metastases or other disease-related causes. The cytopenias led us to question whether the reaction might be an adverse effect of 5-FU. The patient had very high levels of the naturally occurring substrate uracil, which is the natural analogue of fluorouracil. Because these levels were very high, we assumed that the levels of 5-FU were also very high, and that was indeed later proven.

When the patient later consented to a pharmacokinetic protocol and was given a very small test dose of radioactive 5-FU, we were able to demonstrate that the pharmacokinetics were markedly altered and that the 5-FU was essentially not metabolized. We later were able to demonstrate that this patient had no detectable dihydropyrimidine dehydrogenase (DPD) activity.

Studies of the patient's family demonstrated that the father and both the son and daughter had partial DPD activity with a pattern suggesting this to be an autosomal recessive or an autosomal co-dominant trait. These findings convinced us that this was indeed a pharmacogenetic syndrome. Like many pharmacogenetic syndromes, it was characterized by a patient who was completely healthy until exposed to the drug.

At the time, we thought this reaction was a rare event and that we would never see such a case again. Shortly afterward, however, we saw our second case, and then multiple cases since then.

### H&O What is DPD, and how can it impact a patient's response to 5-FU?

**RD** DPD is the first enzyme in the catabolic pathway, which degrades 5-FU. DPD is not only the first step, but also the rate-limiting step. Because it is rate-limiting, 5-FU will accumulate in the presence of DPD deficiency, enabling more anabolism of 5-FU to its active metabolites. Once the excess 5-FU is anabolized, it results in dramatic toxicity not only to tumor cells but also to normal cells. This toxicity is responsible for the adverse effects observed clinically.

Currently, we are comprehensively looking at the *DPYD* gene, which provides instructions for making the DPD enzyme, to better understand the causes of DPD deficiency.

### H&O What are some of the genetic variations in the *DPYD* gene?

**RD** There are several types of genetic variations. Some are single nucleotide polymorphisms (SNPs). The ones that occur in the amino acid coding regions of the gene can lead to formation of a different amino acid that in turn can alter the protein's activity. Variations can occur in the splicing junction, between exons and introns. Others can occur upstream in the promotor and enhancing regions of the genes.

Thus far, we know there are 4 variants that typically are associated with toxicity. These variants are still very rare, and we continue to search for others to try to explain DPD deficiency and develop better and more complete predictors of 5-FU toxicity.

### H&O Which genetic factors alter the expression and function of genes important to 5-FU metabolism?

**RD** There are multiple factors that alter the expression and function of genes involved in 5-FU metabolism.

Some lead to the production of a defective protein. Other variants occur in front of the gene, in the promotor or the enhancer region, and can interfere with transcription.

### H&O What is the goal of your laboratory's research?

**RD** The goal of my laboratory is to develop predictive tests for 5-FU-based chemotherapy to minimize toxicity while maintaining drug efficacy. Ultimately, we would like to be able to individualize 5-FU therapy while at the same time create a screening test for *DPYD* variants.

We have been working toward this goal for more than 2 decades. With the development of genetic tests that are relatively inexpensive, we hope that in a very short period of time, we will be able to comprehensively identify all patients who are likely to develop toxicity from 5-FU before they receive their first dose of the drug.

### H&O Could you please discuss the functional analysis of *DPYD* variants created by your laboratory?

**RD** In the laboratory, we have been able to test whether an individual site-directed mutation that we induce in the *DPYD* gene within a cell line results in decreased DPD activity. In addition to testing for actual enzyme activity, we also test these cells for their response to 5-FU. In doing so, we have been able to associate the specific *DPYD* genotype with the phenotype (DPD activity and 5-FU sensitivity).

### H&O Does your research have implications for other therapies?

**RD** Absolutely. The same type of approach is applicable to many other drugs, not only in the treatment of cancer, but in other therapeutic classes as well.

### Disclosure

*Dr Diasio has no real or apparent conflicts of interest to report.*

### Suggested Readings

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