Dihydropyrimidine Dehydrogenase Deficiency: Impact of Pharmacogenetics on 5-Fluorouracil Therapy

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Abstract

Through the use of pharmacogenetic studies, interindividual variability in response (efficacy and toxicity) to 5-fluorouracil (5-FU) chemotherapy has been linked to the rate-limiting enzyme in the drug's catabolic pathway, known as dihydropyrimidine dehydrogenase (DPD). This pharmacogenetic syndrome, known as "DPD deficiency," results in excessive amounts of 5-FU available to be anabolized to its active metabolites and is relatively undetectable by clinical observation prior to 5-FU administration. Extensive studies have associated both profound and partial deficiency in DPD activity with severe, unanticipated toxicity after 5-FU administration, while research on the molecular basis behind DPD deficiency has been linked to various sequence variants of the *DPYD* gene. Due to the widespread use of 5-FU, the severity of toxicity associated with DPD deficiency, and the prevalence of DPD deficiency in the population, extensive research is continually being performed to develop quick and accurate phenotypic and genotypic assays suitable for clinical settings that would allow clinicians to identify patients susceptible to adverse 5-FU reactions.

Keywords

Pharmacogenetics, DPD deficiency, 5-fluorouracil, drug toxicity, sequence variant, genotype, phenotype

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Introduction: Pharmacogenetics in Cancer Research

Interindividual variability in drug efficacy and toxicity is a consistently and commonly observed occurrence, especially in the heterogeneity of patient response to chemotherapeutic agents. Administration of a standard dose of a given cancer therapy drug to a population of patients produces a range of responses, from no effect to lethal events.[1] These adverse drug reactions due to cancer chemotherapy drastically interfere with health care and are estimated to increase significantly the overall hospital and drug costs.[2] Though numerous clinical variables have been associated with drug response, evidence increasingly shows that genetics is a major determinant of the observed variability in response to therapy. Inherited individual variations in drug-metabolizing enzymes, drug transporters, and drug targets make important contributions to unpredictability in anticancer treatment outcomes, such as efficacy and toxicity, when given at standard doses.[3,4] The study of the inherited molecular basis of interindividual variability in drug response is referred to as pharmacogenetics, a discipline that documents associations between altered drug toxicities and heritable differences in drug metabolism.[5,6] With the completion of the Human Genome Project, pharmacogenetics has produced a conceptual extension called pharmacogenomics, which is broadly defined as the assessment of inherited differences in interindividual drug response using a whole-genome approach. Valuable information gathered from studies in pharmacogenetics and pharmacogenomics on a patient's predicted response to a chemotherapeutic agent may provide the clinician with a greater array of therapeutic options, and it has the potential to lead to the development of individualized therapies.

Oncology is likely to be one of the most appropriate disease areas for the application of pharmacogenetics and pharmacogenomics for several reasons.[7] First, anticancer agents typically display small differences between median therapeutic and toxic doses. Second, many agents undergo a biotransformation and/or detoxification process involving potentially polymorphic enzyme systems involved in its metabolic process. Third, standard dosing procedures of chemotherapeutic agents by body surface area may place patients with altered drug-metabolizing enzyme activity at high risk for toxicity and treatment failure.[6] Taken collectively, these factors

emphasize the clear need for the development of individualized cancer therapy.

Pharmacogenetic studies have been implemented in the study of a specific class of antineoplastic agents known as the fluoropyrimidines; these include the prototype agent 5-fluorouracil (5-FU) and the oral prodrug capecitabine (Xeloda, Roche). 5-FU remains one of the most commonly prescribed anticancer drugs and is used in treating a variety of cancers.[8-10] Its biological activity is mediated through activation or anabolism of the parent compound into cytotoxic nucleotides, which subsequently inhibit thymidylate synthase (TS) and tumor cell replication, while 80–90% of the administered dose is inactivated through catabolism to inactive metabolites (Figure 1).[11,12] Interindividual differences in response, survival, and toxicity among patients treated with standard 5-FU doses have been associated with steps in both the catabolic and anabolic pathways.

The rate-limiting enzyme in the catabolism of 5-FU, dihydropyrimidine dehydrogenase (DPD), converts over 85% of administered 5-FU to its inactive metabolite, 5-fluorodihydrouracil.[13] DPD activity has been shown to have high variability among the general healthy population[14] and has been extensively implicated in 5-FU efficacy and toxicity.[13] For example, in patients with colorectal cancer, low intratumoral expression of DPD has been associated with therapeutic efficacy of 5-FU.[15] However, due to the narrow therapeutic window of 5-FU, 31–34% of patients with

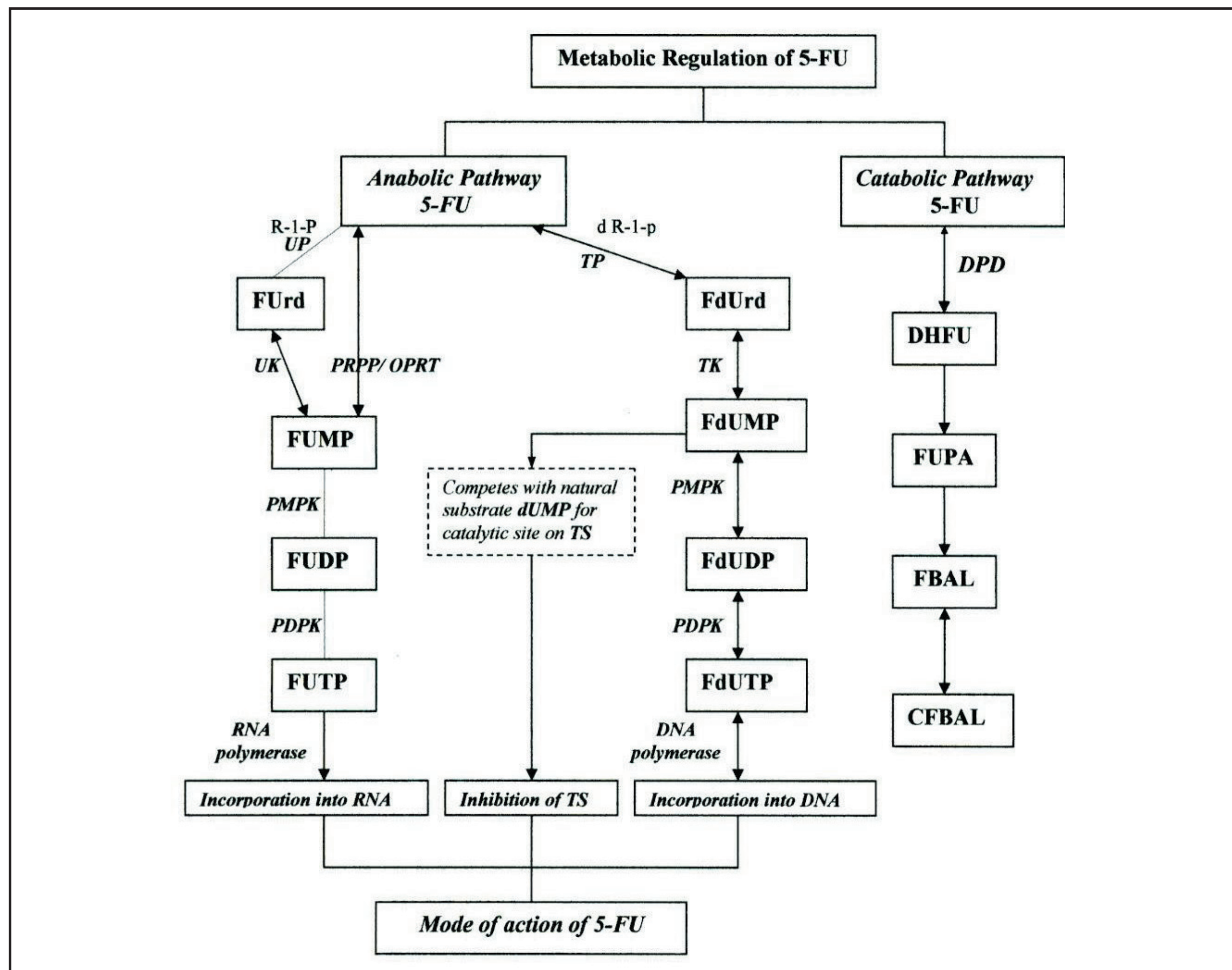

Figure 1. **Metabolic regulation of 5-FU.**

DPD plays an important role in regulating the bioavailability of 5-FU to exert its cytotoxic effect. The anabolic pathway results in incorporation of the active metabolites into RNA and DNA. In addition, the 5-FU generates FdUMP, which competes with deoxyuridine monophosphate (dUMP), the natural substrate of thymidylate synthase, on active catalytic site, preventing the formation of thymidylate, which is essential for DNA synthesis. In the catabolic pathway DPD is essentially responsible for approximately 85% of 5-FU metabolism.

5-FU=5-fluorouracil; R-1-P=ribose-1-phosphate; UP=uridine phosphorylase; Furd=fluorouridine; UK=uridine kinase; PRPP=phosphoribosyl pyrophosphate; OPRT=orotate phosphoribosyltransferase; FUMP=fluorouridine monophosphate; PMPK=pyrimidine monophosphate kinase; FUDP=flurouridne diphosphate; PDPK=pyrimidine diphosphate kinase; FUTP=flurouridne triphosphate; dR-1-P=deoxyribose-1-phosphate; TP=thymidine phosphorylase; FdUrd=fluorodeoxyuridine; TK=thymidine kinase; FdUMP=fluorodeoxyuridine monophosphate; FdUDP=fluorodeoxyuridine diphosphate; FdUTP=fluorodeoxyuridine triphosphate; DPD=dihydropyrimidine dehydrogenase; DHFU=dihydroflurouracil; FUPA=fluroureidopropionic acid; FBAL=*alpha*-fluoro-*beta*-alanine; CFBAL=carboxy-fluoro-beta-alanine.

Adapted from Ezzeldin H, Diasio RB.[37]

colorectal cancer treated with 5-FU displayed dose-limiting grade 3–4 toxicity.[16] In addition, 40–50% of patients experiencing unanticipated 5-FU toxicity have decreased DPD activity[17,18] ranging from partial to complete deficiency. Furthermore, complete or partial loss of the DPD enzyme activity has been extensively associated with defects in the *DPYD* gene that lead to the considerable patient-to-patient variability in therapeutic efficacy and the propensity for 5-FU toxicity.[19,20] These studies collectively define the pharmacogenetic syndrome prevalent in approximately 3% of the population known as "DPD deficiency."[19]

This review focuses on the clinical management of patients treated with 5-FU, as well as the effects of DPD deficiency on the pharmacology of this chemotherapeutic agent. In addition, we will discuss the potential role pharmacogenetic studies and the development of specific genotypic and phenotypic tests can have on improving drug efficacy and safety.

Clinical Diagnosis of DPD Deficiency

Efficacy and toxicity associated with 5-FU therapy have shown significant variation among individuals and have been associated with the activity of the rate-limiting catabolic enzyme DPD. Unanticipated toxic reactions to 5-FU have been linked to both partially and profoundly deficient DPD activity. Several studies have suggested that the identification of DPD deficiency in cancer patients prior to the initiation of 5-FU treatment could predict which patients are at risk for 5-FU toxicity. Unfortunately, DPD deficiency is often undetectable by clinical observation due to the absence of symptoms prior to 5-FU administration, thus making clinical diagnosis by physical observation impossible.

Common clinical observations of a DPD-deficient patient following the administration of 5-FU include fever (secondary to neutropenia), mucositis, stomatitis, and diarrhea.[21-24] The observance of enteric lesions may occur at any level, resulting in a variety of clinical symptoms, such as dysphagia, retrosternal burning, watery diarrhea, and proctitis. The diarrhea can be bloody, and nausea, vomiting, and profuse diarrhea can lead to extreme cases of dehydration and hypotension.[25] Disruption of gut-lining integrity may permit access of enteric organisms into the bloodstream, with the potential for overwhelming sepsis, particularly if the granulocyte nadir coincides with diarrhea. Other physical signs may include changes to the skin, typically as a maculopapular rash or increased pigmentation.

Other symptoms observed in DPD-deficient patients suffering from 5-FU toxicity may include neurological abnormalities, such as changes in cognitive function, cerebellar ataxia, and the presence of a broad-based gait.[23,25] However, these manifestations are often subtle and can go unrecognized unless specifically sought during a complete neurologic examination. Severe effects of 5-FU toxicity on the level of consciousness reaching a comatose state may also be observed.[23]

One of the more striking examples of severe 5-FU toxicity due to DPD deficiency reported by our group involved the topical application of 5% 5-FU cream to the scalp of a 76-year-old male patient with basal cell carcinoma.[26] It was the first example of severe toxicity associated with the topical use of 5-FU and was shown to be the result of virtually undetectable DPD enzymatic activity in the patient's peripheral blood mononuclear cells. Given the generally accepted safety of topical 5-FU use, this particular case represents an important example indicating that severe toxicity may be associated with even minor exposure to 5-FU in patients with severe DPD deficiency.[27]

Routine blood investigations performed at the time of clinical manifestation of 5-FU toxicity may indicate abnormalities, such as leukopenia, thrombocytopenia, and anemia, manifested as significant decreases in the absolute neutrophil count (near 0). However, routine chemistry tests and urine analysis are often normal, adding to the difficulty of clinical diagnosis of DPD deficiency.

Patient Management

There are few options for managing patients' toxicities even when DPD deficiency is suspected soon after treatment with 5-FU. Numerous approaches have been suggested for managing toxicities in suspected or diagnosed DPD-deficient patients.[23] The first and most obvious action would be to stop any further administration of 5-FU or related drug, followed by aggressive supportive care. For example, antibiotic and antibacterial coverage may be used in treating potential bacterial and fungal infections resulting from the invasion of enteric organisms through the weakened gut lining. Dehydration and hypotension may be treated with appropriate fluid and electrolyte support. In the most severe cases, hospitalization in the intensive care unit may be necessary.

When dealing with drug toxicities, elimination of the unreacted drug remaining in the body would be advantageous. This has commonly been performed with other drugs by hemodialysis and hemoperfusion. Unfortunately, with 5-FU the effectiveness of either method is limited even though it is a small molecular-weight molecule capable of passing through dialysis membranes, due to the fact that 5-FU is rapidly cleared through the urine, even in the presence of complete DPD deficiency.[23] Thus by the time DPD deficiency is suspected it is typically too late to obtain benefit from hemodialysis and hemoperfusion.

To overcome the block in thymidylate synthesis from the inhibition of thymidylate synthase by the 5-FU nucleotide fluorodeoxyuridine monophosphate (FdUMP), pyrimidine nucleosides (thymidine or uridine) may be quickly administered soon after 5-FU administration.[11] The use of colony-stimulating growth factor to overcome the associated febrile neutropenia or agranulocytosis has also been suggested as a means of managing DPD deficiency.[23] Although it has been used in the management of several cases, its benefit is still unproven and may in fact be associated with adverse consequences.

Laboratory Diagnosis of DPD Deficiency

Elevated levels of uracil and/or thymine (the natural substrates

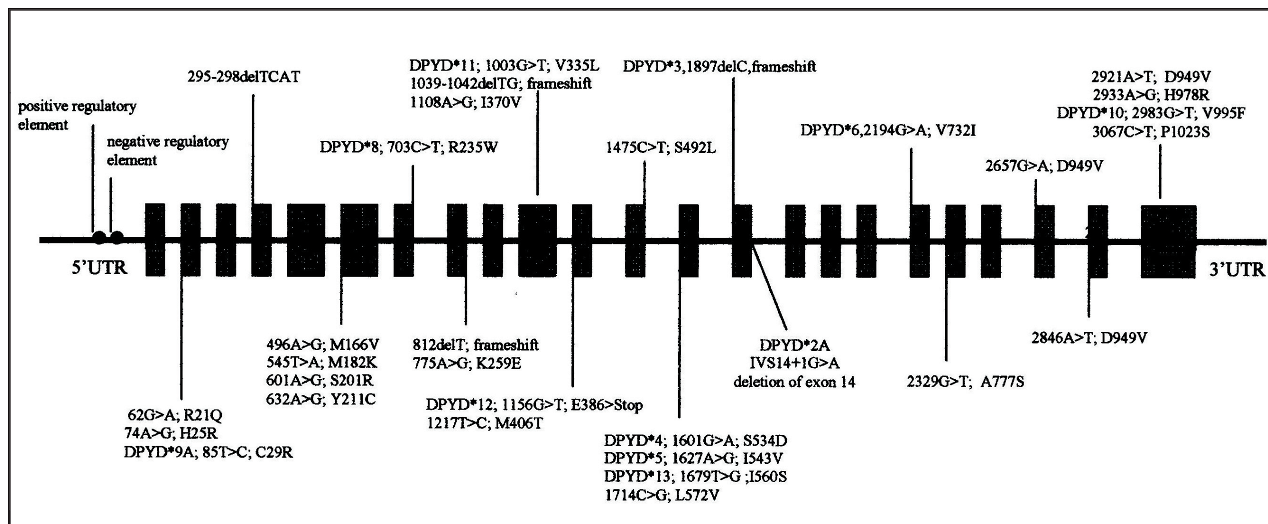

Figure 2. **Sequence variations identified in the DPYD gene.**

This figure locates the sequence variations identified in the coding and noncoding regions of the *DPYD* gene. The location of the positive and negative regulatory elements identified 5'UTR, upstream of the transcription start point is shown on the figure. Other sequence variations that were reported but not shown on the figure include: DPYD*9B, which is composed of both sequence variants DPYD*9A (85T>C; C29R) in exon 2 and 2846A>T (D949V) in exon 23; and DPYD*2B, which is composed of both sequence variants DPYD*2A (IVS14+1G>A) in intron 14 and DPYD*5 (1627A>G; I543V) in exon 13.

for DPD) in the plasma or urine is indicative of complete or profound DPD deficiency and can be detected using a variety of methods.[28] However, these methods are not suitable for detecting partial DPD deficiency, which is more prevalent in the population. The radioenzymatic assay that directly measures DPD activity is the standard and most accurate method used in detecting partial DPD deficiency.[17] DPD activity may be determined via analysis of peripheral blood mononuclear (PBM) cells. PBM cells are quite suitable for DPD activity assessment due to the absence of subsequent enzymes of the pyrimidine catabolic pathway.[8] Although the assay is sensitive and accurate, it is time consuming and labor intensive, which discourages its use in screening for DPD-deficient patients prior to 5-FU therapy in most clinical settings.

Over the past few years, more attempts have been made to develop both user- and patient-friendly assays for the screening of DPD deficiency prior to 5-FU administration. One such attempt includes antibody-based tests, using polyclonal or monoclonal antibodies to DPD.[29,30] Though they typically permit diagnosis of completely DPD-deficient individuals, they are somewhat less accurate in assessing partially deficient individuals (typically individuals with a heterozygous mutation). Since the vast majority of patients are partially deficient, these assays are of limited use.

Recently, a uracil breath test (UBT) has been developed that permits the rapid diagnosis of both profound and partial DPD deficiency (≤90 minutes) before the administration of 5-FU and allows differentiation among individuals with normal-range DPD activity.[31] This noninvasive and cost-effective assay uses a stable isotope (^{13}C) localized at the 2-carbon of uracil and can differentiate between individuals with normal DPD activity and those with partial DPD deficiency. Rapid and efficient analysis along with its user-friendly and noninvasive qualities make the uracil breath test a leading candidate for future general use.

Genetic Testing for DPD Deficiency

The gene encoding for the DPD enzyme (*DPYD*) has been extensively studied to elucidate the molecular basis of DPD deficiency. The human *DPYD* gene is located on chromosome 1p22 and consists of 23 exons.[32,33] It is at least 950 kb in length with 3 kb of coding sequence and an average intron size of approximately 43 kb.[34] More recently, the promoter region of the gene has been cloned and characterized.[35] This information led to the examination of the cDNA from individuals with DPD deficiency to determine if any obvious structural abnormalities could explain the dysfunctional DPD protein. By identifying these mutations in the *DPYD* gene which lead to DPD deficiency, rapid screening techniques can be used to identify individuals with these specific mutations to determine the susceptibility to 5-FU toxicity prior to treatment. Currently, there are over 30 reported mutations located in both the coding and noncoding regions of the *DPYD* gene (none in the promoter region) (Figure 2).[20,36-42] Familial studies of both normal and deficient members have also been used to provide valuable information on whether the particular sequence variant results in a functional change in vivo as well as determining the inheritance pattern of DPD deficiency. Numerous studies genotyping DPD deficient patients, their family members, and healthy individuals have shown that the splice-site mutation (IVS14+1G>A) known as DPYD*2A is the most characterized and frequently observed allele associated with decreased DPD enzyme activity.[37,41] This G-to-A splice-site mutation of intron 14 leads to the skipping of exon 14 in the process of DPD pre-mRNA splicing. The resulting mature DPD mRNA lacks a 165-nucleotide segment encoding the amino

acids 581–635. Studies have shown complete loss of DPD activity with homozygous DPYD*2A, while the heterozygous DPYD*2A mutation resulted in partial DPD deficiency.[26,20] Another mutation associated with DPD deficiency is a single amino acid substitution known as DPYD*13 (1679T>G; I560S). Though the substitution does not occur in any recognized functional domain, the I560 position shows 100% conservation across 5 mammalian species (human, mouse, rat, bovine, and pig),[42] suggesting that this position is important in maintaining DPD enzyme activity. The correlation between DPYD*13 and DPD deficiency was most evident in the case of a profoundly deficient patient with heterozygous mutations for both DPYD*13 and DPYD*2A. Screening of the patient's 2 partially deficient children and spouse with normal activity revealed an autosomal codominant pattern of inheritance with 1 child containing the heterozygous DPYD*2A mutation, while the other showed the heterozygous DPYD*13 mutation.[28]

Though conflicting results correlating specific genotypes to a DPD-deficient phenotype have been presented,[43] the high incidence of DPYD*2A and the association of specific mutations with DPD deficiency warranted the development of numerous quick screening assays for these specific sequence variations.

Screening techniques for the known mutations of the *DPYD* gene include allele specific polymerase chain reaction (ASPCR), restriction fragment length polymorphism (RFLP), and pyrosequencing.[26,41,44] These methods offer rapid and high-throughput screening capabilities; however, they can only be used to screen for the known mutations. Denaturing high-performance liquid chromatography (DHPLC) has the advantage of detecting both known and unknown sequence variations.[45] While these rapid genetic screening techniques require a minimal amount of starting material from the patient, the equipment necessary to perform the analysis is expensive and may not be suitable for the general clinical setting. Although direct sequencing remains the standard method for the detection and identification of both known and unknown sequence variations, it is expensive, time-consuming, and labor-intensive.

Conclusion

Adverse drug reactions are a severe concern to both health care practitioners and the general public, accounting for over 100,000 fatalities per year; it is the fourth leading cause of death in the United States, after heart disease, cancer, and stroke.[46] It is now clear that genetic differences in drug metabolizing enzymes, transporters, or receptors may result in altered drug response—including both efficacy and toxicity. The small difference between the mean efficacious dose and the mean toxic dose make chemotherapeutic agents prime candidates for the occurrence of interindividual variability in drug efficacy and toxicity. Pharmacogenetic studies on chemotherapeutic agents have the potential to revolutionize the way clinicians determine a patient's optimal treatment regimen through the prediction of an individual's responsiveness to a particular therapy. In the case of fluoropyrimidine chemotherapy, screening for DPD deficiency may provide predictive insight into an individual's susceptibility to unanticipated 5-FU toxicity before the initiation of treatment. Patients with a determined deficiency of DPD might be selected for alternative treatment modalities containing nonfluoropyrimidine compounds. For example, irinotecan, oxaliplatin, and raltitrexed have been safely applied in the treatment of a patient suffering from partial DPD deficiency.[47]

5-FU pharmacogenetic studies have aided in the advancement of predicting patients' response to 5-FU chemotherapy through the development of both phenotypic and genotypic assays for DPD. Though many of these phenotypic tests are not yet suitable for widespread clinical use, the recently reported UBT has shown the greatest potential for clinical diagnosis of DPD deficiency due to its quick operation, efficient measurement, and user-friendly and noninvasive qualities.[31]

Although information about the DPD enzyme and its coding gene (*DPYD*) is rapidly accumulating, future studies are still required to fully elucidate the molecular basis of DPD deficiency and to resolve the discrepancies in genotype-to-phenotype correlations. Further studies on other enzymes of the fluoropyrimidine metabolic pathway in patients with an unknown molecular basis for unanticipated 5-FU toxicity may reveal other attributing factors. For example, variability in response to 5-FU has been linked to thymidylate synthase (TS), where drug resistance and poor prognosis were associated with overexpression of this enzyme and a previously identified polymorphism in the 5'UTR in the enhancer region (TSER) of this gene.[48,49] Deficiencies in the dihydropyrimidinase and ureidopropionase enzymes (the subsequent enzymes in the catabolic pathway of 5-FU following DPD) have also been implicated in unexpected 5-FU toxicity.[50,51] Taken collectively, these factors could provide a greater understanding of the basis behind unanticipated 5-FU toxicity and potentially be used in the development of future diagnostic tests to provide a more accurate determination of a patient's responsiveness to 5-FU treatment.

References

1. Watters JW, McLeod HL. Cancer pharmacogenomics: current and future applications. *Biochemica et Biophysica Acta.* 2003;1603:99-111.
2. Wiffen P, Gill M, Edwards J, Moore A. Adverse drug reactions in hospital patients: a systemic review of the prospective and retrospective studies. *Bandolier Extra.* (2002) Available at: http://www.jr2.ox.ac.uk/bandolier/extra.html.
3. Desai AA, Innocenti F, Ratain MJ. Pharmacogenomics: road to anticancer therapeutics nirvana? *Oncogene.* 2003;22:6621-6628.
4. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science.* 1999;286:487-491.
5. Nagasubramanian R, Innocenti F, Ratain MJ. Pharmacogenetics in cancer treatment. *Annu Rev Med.* 2003;54:437-452.
6. Evans DA. Genetic variations in the acetylation of isoniazid and other drugs. *Ann N Y Acad Sci*. 1968;151:723-733.
7. Veenstra DL, Higashi MK. Assessing the cost-effectiveness of pharmacogenomics. *AAPS Pharmsci.* 2000;2:1-11.
8. Diasio RB. Dihydropyrimidine dehydrogenase (DPD) deficiency: a 5-fluorouracil-associated pharmacogenomic syndrome. *Horiz Cancer Ther.* 2003;4;1:9-21.
9. *Scrip's Cancer Chemotherapy Report, Scrip World Pharmaceutical News.* London: PJB Publications Ltd; 2002.

10. Diasio RB, Lu Z, Zhang R, Shahinian H. Fluoropyrimidine catabolism. In: Muggia FM, ed. *Concepts, Mechanisms, and New Targets for Chemotherapy.* Boston, Mass: Kluwer Academic Publishers; 1995:71-94.
11. Daher GC, Harris BE, Diasio RB. Metabolism of pyrimidine analogues and their nucleosides. *Metabolism and Reactions of Anticancer Drugs, Volume 1: The International Encyclopedia of Pharmacology and Therapeutics.* Chap. 2, Oxford, England: Pergamon Press; 1994.
12. Diasio RB, Harris BE. Clinical pharmacology of 5-FU. *Clin Pharmacokinet.* 1989;16:215-237.
13. Heggie GD, Sommadossi JP, Cross DS, et al. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res.* 1987;47:2203-2206.
14. Lu Z, Zhang R, Diasio RB. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified patients, and clinical implification in 5-fluorouracil chemotherapy. *Cancer Res*. 1993;53:5433-5438.
15. Salonga D, Danenberg KD, Johnson M, et al. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res*. 2000;6:1322-1327.
16. Meta-Analysis Group in Cancer. Toxicology of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. *J Clin Oncol*. 1998;16:3537-3541.
17. Johnson MR, Yan J, Shao L, et al. Semi-automated radioassay for determination of dihydropyrimidine dehydrogenase (DPD) activity: screening cancer patients for DPD deficiency, a condition associated with 5-fluorouracil toxicity. *J Chromatogr B Biochem Sci Appl.* 1997;696:183-191.
18. Johnson MR, Diasio RB. Importance of dihydropyrimidine dehydrogenase (DPD) deficiency in patients exhibiting toxicity following treatment with 5-fluorouracil. *Adv Enzyme Regul* 2001;41:151-157.
19. Lu Z, Zhang R, Carpenter JT, Diasio RB. Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: implication for 5-fluorouracil-based chemotherapy. *Clin Cancer Res*. 1998;2:325-329.
20. Wei X, McLeod HL, McMurrough J, et al. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. *J Clin Invest*. 1996;98:610-615.
21. Diasio RB, Beavers TL, Carpenter JT. Familial deficiency of dihydropyrimidine dehydrogenase: biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. *J Clin Invest.* 1988;81:47-51.
22. Harris BE, Carpenter JT, Diasio RB. Severe 5-fluorouracil toxicity secondary to dihydropyrimidine dehydrogenase deficiency: a potentially more common pharmacogenetic syndrome. *Cancer.* 1991;68:499-501.
23. Morrison GB, Bastian A, Dela Rosa T, et al. Dihydropyrimidine dehydrogenase (DPD) deficiency: a pharmacogenetic defect causing severe adverse reactions to 5-fluorouracil-based chemotherapy. *Oncol Nurs Forum.* 1997;24:83-88.
24. Grem JL. 5-fluoropyrimidines. In: Chabner BA, Longo DL, eds. *Cancer Chemotherapy and Biotherapy 2nd ed.* Philadelphia: Lippincott-Raven; 1996:149-203.
25. Takimoto CH, Lu Z-H, Zhang R, et al. Severe neurotoxicity following 5-fluouracil-based chemotherapy in a patient with dihydropyrimidine dehydrogenase deficiency. *Clin Cancer Res.* 1996;2:477-481.
26. Johnson MR, Hageboutros A, Wang K, et al. Life threatening toxicity in a dihydropyrimidine dehydrogenase-deficient patient following treatment with topical 5-fluorouracil. *Clin Cancer Res.* 1999;5:2006-2011.
27. Allegra C. Dihydropyrimidine dehydrogenase activity: prognostic partner of 5-fluorouracil? *Clin Cancer Res.* 1999;5:1947-1949.
28. Johnson MR, Wang K, Diasio RB. Profound dihydropyrimidine dehydrogenase (DPD) deficiency resulting from a novel compound heterozygote genotype. *Clin Cancer Res.* 2002;8:768-774.
29. Diasio RB, Van Kuilenburg AB, Lu Z, et al. Determination of dihydropyrimidine dehydrogenase (DPD) in fibroblasts of a DPD deficient pediatric patient and family members using a polyclonal antibody to human DPD. *Adv Exp Med Biol.* 1994;370:7-10.
30. Honda T, Inagawa H, Fukushima M, et al. Development and characterization of a monoclonal antibody with cross-reactivity towards uracil and thymine, and its potential use in screening patients treated with 5-fluorouracil for possible risks. *Clin Chim Acta*. 2002;322:59-66.
31. Mattison LK, Ezzeldin H, Carpenter M, et al. Rapid identification of dihydropyrimidine dehydrogenase deficiency by using a novel 2-^{13}C-uracil breath test. *Clin Cancer Res*. 2004;10:2652-2658.
32. Takai S, Fernandez-Salguero P, Kimura S, et al. Assignment of the human dihydropyrimidine dehydrogenase gene (DPYD) to chromosome region 1p22 by fluorescence in situ hybridization. *Genomics*. 1994;24:613-614.
33. Johnson MJ, Wang K, Tillmans S, et al. Structural organization of the human dihydropyrimidine dehydrogenase gene. *Cancer Res.* 1997;57:1660-1663.
34. Wei X, Elizondo G, Sapone A, et al. Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics*. 1998;51:391-400.
35. Shestopol SA, Johnson MR, Diasio RB. Molecular cloning and characterization of the human dihydropyrimidine dehydrogenase gene. *Biochim Biophys Acta*. 2000;1494:162-169.
36. Van Kuilenburg ABP, Vreken P, Abeling NGGM, et al. Genotype and phenotype in patients with dihrdropyrimidine dehydrogenase deficiency. *Hum Genet.* 1999;104:1-9.
37. Ezzeldin H, Diasio RB. Pharmacogenetics and cancer chemotherapy. In: Budman DR, Calvert H, Rowinsky E, eds. *Handbook of Anticancer Drug Development*. Philadelphia: Lippincott Williams and Wilkins; 2003:343-378.
38. Van Kuilenburg ABP. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer*. 2004;40:939-950.
39. Albin N, Johnson MR, Shahinian H, et al. Initial characterization of the molecular defect in human dihydropyrimidine dehydrogenase deficiency. *Proc Am Assoc Cancer Res.* 1995;36:211A.
40. Meinsma R, Fernandez-Salguero P, Van Kuilenburg AB, et al. Human polymorphism in drug metabolism: mutation in the dihydropyrimidine dehydrogenase gene results in exon skipping and thymine uraciluria. *DNA Cell Biol.* 1995;14:1-6.
41. Van Kuilenburg ABP, Muller EW, Haasjes J, et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14+1G>A mutation causing DPD deficiency. *Clin Cancer Res*. 2001;7:1149-1153.
42. Mattison LK, Johnson MR, Diasio RB. A comparative analysis of translated dihydropyrimidine dehydrogenase cDNA; conservation of functional domains and relevance to genetic polymorphisms. *Pharmacogenetics*. 2002;12:133-144.
43. Collie-Duguid ES, Etienne MC, Milano G, McLeod HL. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics.* 2000;10:217-23.
44. Ahluwalia R, Freimuth R, McLeod HL, Marsh S. Use of pyrosequencing to detect clinically relevant polymorphisms in dihydropyrimidine dehydrogenase. *Clin Chem.* 2003;10:1661-1664.
45. Ezzeldin H, Okamoto Y, Johnson MR, Diasio RB. A high-throughput denaturing high performance liquid chromatography method for the identification of variant alleles associated with dihydropyrimidine dehydrogenase deficiency. *Ann Biochem*. 2002;306:63-73.
46. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA*. 1998;279:1200-1205.
47. Volk J, Reinke F, van Kuilenburg ABP, et al. Safe administration of irinotecan, oxaliplatin and raltitrexed in a DPD-deficient patient with metastatic colon cancer. *Ann Oncol.* 2001;12:569-571.
48. Johnston PG, Lenz HJ, Leichman CG, et al. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. *Cancer Res*. 1995;55:1407-1412.
49. Pullarkat ST, Stoehlmacher J, Ghaderi V, et al. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics J*. 2001;1:65-70.
50. van Kuilenburg AB, Meinsma R, Zonnenberg BA, et al. Dihydropyrimidinase deficiency and severe 5-fluorouracil toxicity. *Clin Cancer Res*. 2003;9:4363-4367.
51. Ohse M, Matsuo M, Ishida A, Kuhara T. Screening and diagnosis of beta-ureidopropionase deficiency by gas chromatographic/mass spectrometric analysis of urine. *J Mass Spectrom*. 2002;37:954-962.