Increased Prevalence of Dihydropyrimidine Dehydrogenase Deficiency in African-Americans Compared with Caucasians

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Abstract

Purpose: African-American patients with colorectal cancer were observed to have increased 5-fluorouracil (5-FU)–associated toxicity (leukopenia and anemia) and decreased overall survival compared with Caucasian patients. One potential source for this disparity may be differences in 5-FU metabolism. Dihydropyrimidine dehydrogenase (DPD), the initial and rate-limiting enzyme of 5-FU catabolism, has previously been shown to have significant interpatient variability in activity. Several studies have linked reduced DPD activity to the development of 5-FU toxicity. Although the distribution of DPD enzyme activity and the frequency of DPD deficiency have been well characterized in the Caucasian population, the distribution of DPD enzyme activity and the frequency of DPD deficiency in the African-American population are unknown.

Experimental Design: Healthy African-American (n = 149) and Caucasian (n = 109) volunteers were evaluated for DPD deficiency using both the [2-^{13}C]uracil breath test and peripheral blood mononuclear cell DPD radioassay.

Results: African-Americans showed significantly reduced peripheral blood mononuclear cell DPD enzyme activity compared with Caucasians (0.26 $\pm$ 0.07 and 0.29 $\pm$ 0.07 nmol/min/mg, respectively; P = 0.002). The prevalence of DPD deficiency was 3-fold higher in African-Americans compared with Caucasians (8.0% and 2.8%, respectively; P = 0.07). African-American women showed the highest prevalence of DPD deficiency compared with African-American men, Caucasian women, and Caucasian men (12.3%, 4.0%, 3.5%, and 1.9%, respectively).

Conclusion: These results indicate that African-Americans, particularly African-American women, have significantly reduced DPD enzyme activity compared with Caucasians, which may predispose this population to more 5-FU toxicity.

5-Fluorouracil (5-FU) and its fluoropyrimidine derivatives (e.g., capecitabine) are widely prescribed in oncologic practice to treat gastrointestinal malignancies and are often used in the management of breast and head and neck cancer (1–4). However, despite its widespread use, ~31% of patients with advanced colorectal cancer who receive bolus 5-FU regimens experience grades 3 to 4 hematologic toxicities (5). The pharmacogenetic syndrome, dihydropyrimidine dehydrogenase (DPD; EC 1.3.1.2) deficiency, has been shown to predispose cancer patients to severe 5-FU toxicity (6–9). In particular, it is estimated that 40% to 60% of patients with cancer who present with severe 5-FU toxicity are DPD-deficient (10, 11).

Several studies show the pivotal role of DPD in 5-FU metabolism and response. Earlier biochemical studies showed that DPD, the initial and rate-limiting enzyme of the pyrimidine catabolic pathway, degrades uracil, thymine, and 5-FU to dihydrouracil, dihydrothymine, and 5-fluoro-dihydrouracil, respectively (12, 13). Pharmacokinetic evaluation has further shown that DPD catabolizes >80% of an administered dose of 5-FU, thereby determining the amount of 5-FU available for anabolism (7). Furthermore, data from combined pharmacokinetic/pharmacodynamic studies in cancer patients show that reduced DPD enzyme activity (DPD deficiency) is associated with decreased 5-FU clearance, and increased 5-FU area under the curve, exposure, and toxicity (7, 14, 15).

Population studies by our laboratory and others have shown that ~3% to 5% of the Caucasian population is DPD-deficient (2, 9, 16). Whereas the frequency of this pharmacogenetic syndrome in the general population suggests that routine screening for DPD deficiency should be done prior to 5-FU administration to cancer patients, the technical complexity of the available genotypic and phenotypic assays limit application to retrospective analysis of patients subsequent to the development of 5-FU toxicity (17, 18). To address this problem, we

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recently developed a clinically feasible oral [2-^{13}C]uracil breath test (UraBT), which detected DPD deficiency with 100% sensitivity and 96% specificity in <1 hour (18). More recently, we validated the UraBT by evaluating plasma [2-^{13}C]uracil, [2-^{13}C]dihydrouracil, and breath $^{13}CO_2$ pharmacokinetics in subjects with normal DPD enzyme activity as well as DPD deficiency. We showed that UraBT $^{13}CO_2$ concentrations are significantly correlated with DPD enzyme activity and plasma [2-^{13}C]uracil clearance and [2-^{13}C]dihydrouracil formation (19). These results suggest that the UraBT may be used to rapidly assess variability in *in vivo* pyrimidine catabolism.

Several clinical studies have compared toxicity and survival among African-American and Caucasian patients with colorectal cancer receiving 5-FU regimens. In particular, McCollum and colleagues observed increased toxicity (leukopenia and anemia) in African-American patients with colorectal cancer compared with Caucasian patients with colorectal cancer, whereas Govindarajan and colleagues observed decreased survival in African-American patients with colorectal cancer compared with Caucasian patients (20–23). One potential source for this disparity in response may be differences in 5-FU metabolism, particularly 5-FU catabolism, between African-Americans and Caucasians. Notably, the distribution of DPD enzyme activity and frequency of DPD deficiency in the African-American population has not been characterized. A recent study of peripheral blood mononuclear cell (PBMC) DPD enzyme activity from 150 Japanese subjects and our previous study of an East Indian cohort using the UraBT suggest that racial differences may be present in the distribution of DPD enzyme activity and frequency of DPD deficiency (1, 24). In the current study, we used the UraBT to screen a population of healthy African-American and Caucasian volunteers for DPD deficiency. We examined (*a*) the distribution of PBMC DPD enzyme activity in African-Americans versus Caucasians, (*b*) the distribution of the UraBT DOB_{50} (the concentration of $^{13}CO_2$ in the breath 50 minutes after [2-^{13}C]uracil administration) in African-Americans versus Caucasians, (*c*) the frequency of DPD deficiency in African-Americans versus Caucasians, and (*d*) gender differences in the frequency of DPD deficiency.

Subjects and Methods

Subjects. One hundred and forty-nine healthy African-American volunteers (73 African-American women and 76 African-American men) and 109 healthy Caucasian volunteers (57 Caucasian women and 52 Caucasian men) participated in this Institutional Review Board–approved protocol at the University of Alabama Hospital's General Clinical Research Center. Volunteers <19 years of age were ineligible for participation. Volunteers were also excluded from the study if they had respiratory, gastric, or metabolic diseases.

DPD radioassay. The DPD radioassay was done as described in greater detail elsewhere (25). To minimize variations resulting from a known circadian rhythm in DPD enzyme activity (26), 60 mL of whole blood was collected at approximately 12:00 p.m. from each subject on the same day as their UraBT. PBMCs were isolated, suspended in buffer A (35 mmol/L potassium phosphate, 2.5 mmol/L magnesium chloride, and 10 mmol/L 2-mercaptoethanol; pH 7.4), and lysed by sonication. The protein concentration of PBMC cytosol was determined by a Bradford assay (27). Approximately 250 μg of total protein was added to a reaction mixture containing 200 μmol/L of NADPH, buffer A, and 8.2 μmol/L of [6-^{14}C] 5-FU (56 mCi/mmol) and incubated at 37°C for 30 minutes. One hundred and thirty–microliter aliquots of the reaction mixture were removed every 5 minutes and immediately placed into termination tubes containing an equal volume of ice-cold ethanol. Protein was precipitated by incubating the mixture at −80°C overnight. The mixture was then thawed and filtered. [6-^{14}C]-FUH_2 and [6-^{14}C]-5-FU were separated by reverse phase high-pressure liquid chromatography and quantified using previously described methods (25). The amount of [6-^{14}C]-FUH_2 formed at each time point (y axis) was plotted against time (x axis), and the formation rate of [6-^{14}C]-FUH_2 was computed. DPD enzyme activity was determined by dividing the [6-^{14}C]-FUH_2 formation rate by the amount of total protein added to the reaction mixture. Based on previous population studies done in our laboratory, volunteers were considered to have DPD enzyme activity in the reference range when their fresh PBMC DPD enzyme activity was ≥0.18 nmol/min/mg protein (95% distribution range), partial DPD deficiency when their fresh PBMC DPD enzyme activity was <0.18 but ≥0.10 nmol/min/mg protein (99% distribution range), profound DPD deficiency when their fresh PBMC DPD enzyme activity was <0.10 but ≥0.00 nmol/min/mg protein (outside of the lower limit of the 99% distribution range), and complete DPD deficiency when PBMC DPD enzyme activity was undetectable (2, 9, 25).

Uracil breath test. The UraBT principle and methodology is described in greater detail elsewhere (18). To minimize variation resulting from a known circadian rhythm in DPD enzyme activity, the UraBT protocol commenced at 8:00 a.m. (26). Baseline breath samples were collected from overnight fasting volunteers in three 1.2 L bags (Otsuka Pharmaceuticals, Tokushima, Japan). An aqueous solution of 6 mg/kg of [2-^{13}C]uracil (Cambridge Isotope Laboratories, Andover, MA) was ingested, and 21 breath samples were collected in 300 mL bags (Otsuka Pharmaceuticals) over 180 minutes post-[2-^{13}C]uracil ingestion. The concentration of $^{13}CO_2$ in post-dose breath samples, reported in delta over baseline (DOB) notation, was measured using IR spectrophotometry (UBiT-IR_{300}; Meretek Diagnostics, Lafayette, CO). The breath profile for each subject was constructed by graphing the concentration of $^{13}CO_2$ in breath versus time (y and x axes, respectively). UraBT indices [T_{max}, C_{max}, DOB_{50}, and PDR_{180} (percentage of the [2-^{13}C]uracil dose recovered in the breath as $^{13}CO_2$ over 180 minutes post-[2-^{13}C]uracil ingestion)] were determined as previously described (18, 28). Based on our previously published analysis, the UraBT DOB_{50} was determined to optimally identify volunteers with DPD deficiency (18). UraBT DOB_{50} values <128.9 DOB categorized volunteers as having DPD deficiency (18). Alternatively, UraBT DOB_{50} values ≥128.9 DOB categorized volunteers as having normal DPD enzyme activity (18).

Statistical analysis. Mean values of continuous outcomes (i.e., PBMC DPD enzyme activity and UraBT DOB_{50}) were computed for each gender and race combination (i.e., Caucasian men, Caucasian women, African-American men, and African-American women). Mean values were compared for each subgroup using generalized linear models with race, gender, and the interaction of race and gender covariates as predictors. The least square means and 95% confidence intervals were also computed. The prevalence of DPD deficiency was computed as the proportion of individuals demonstrating reduced PBMC DPD enzyme activity in each race and gender subgroup. The prevalence of DPD deficiency among gender and racial groups was compared using the χ^2 or Fishers exact test. The odds ratio and 95% confidence intervals were also computed. All analyses were conducted with SAS version 9.1. For all analyses, $P < 0.05$ was deemed as statistically significant.

Results

Distribution of PBMC DPD enzyme activity. PBMC DPD enzyme activity from the entire study population ($n = 258$) was normally distributed. The mean (±SE) DPD enzyme activity observed for the entire study population ($n = 258$) was 0.27 ± 0.004 nmol/min/mg. Of the 15 volunteers that showed DPD

deficiency (<0.18 nmol/min/mg), 11 volunteers showed partial deficiency and 4 volunteers showed profound DPD deficiency. No volunteers showed complete DPD deficiency.

Distribution of PBMC DPD enzyme activity in the African-American and Caucasian population. The distributions of PBMC DPD enzyme activity observed in healthy African-American (n = 149) and Caucasian (n = 109) volunteers were both normally distributed (Fig. 1). The distribution of DPD enzyme activity in the African-American population was negatively skewed (with the tail extending into the low range of DPD enzyme activity), whereas the distribution in the Caucasian population was positively skewed (with the tail extending into the high range of DPD enzyme activity). The coefficient of skewness was −0.10 and 0.72, respectively.

African-American volunteers had significantly lower DPD enzyme activity compared with Caucasian volunteers (P = 0.002). The mean (±SE) DPD enzyme activity observed for African-Americans and Caucasians was 0.26 ± 0.006 and 0.29 ± 0.007 nmol/min/mg, respectively.

Prevalence of DPD deficiency in the healthy African-American and Caucasian population. The prevalence of DPD deficiency was 3-fold greater in the African-American population compared with the Caucasian population (P = 0.07). The prevalence of DPD deficiency in the African-American population was 8.0%, with 12 of 149 volunteers demonstrating DPD deficiency. Four of the 12 DPD-deficient African-American volunteers showed profound DPD deficiency, whereas 8 of the 12 showed partial DPD deficiency. The prevalence of DPD deficiency in the Caucasian population was 2.8%, with 3 of 109 volunteers demonstrating partial DPD deficiency.

PBMC DPD enzyme activity and prevalence of DPD deficiency in African-American women. Stratification of volunteers by gender showed that women had significantly lower DPD enzyme activity compared with men (0.25 ± 0.006 and 0.29 ± 0.006, respectively; $P \leq 0.001$). Further stratification of the volunteers by race and gender showed that African-American women had a significantly lower DPD enzyme activity (0.24 ± 0.008 nmol/min/mg) compared with African-American men (0.28 ± 0.008 nmol/min/mg), Caucasian women (0.28 ± 0.008 nmol/min/mg), Caucasian men (0.30 ± 0.01 nmol/min/mg; $P \leq 0.003$ for each pairwise comparison). African-American women were also observed to have the highest prevalence of DPD deficiency (12.3% with 9 of 73 volunteers demonstrating DPD deficiency), compared with African-American men (4.0% with 3 of 76 volunteers demonstrating DPD deficiency; P = 0.08), Caucasian women (3.5% with 2 of 57 volunteers demonstrating DPD deficiency; P = 0.12), and Caucasian men (1.9% with 1 of 52 volunteers demonstrating DPD deficiency; P = 0.09). Three of the nine DPD-deficient African-American women showed profound DPD deficiency whereas the remaining six volunteers showed partial DPD deficiency. One of the three DPD-deficient African-American men showed profound DPD deficiency whereas the remaining two showed partial DPD deficiency. All cases of DPD deficiency observed in Caucasian men and women were partial DPD deficiency; no profound DPD deficiency was observed in Caucasians.

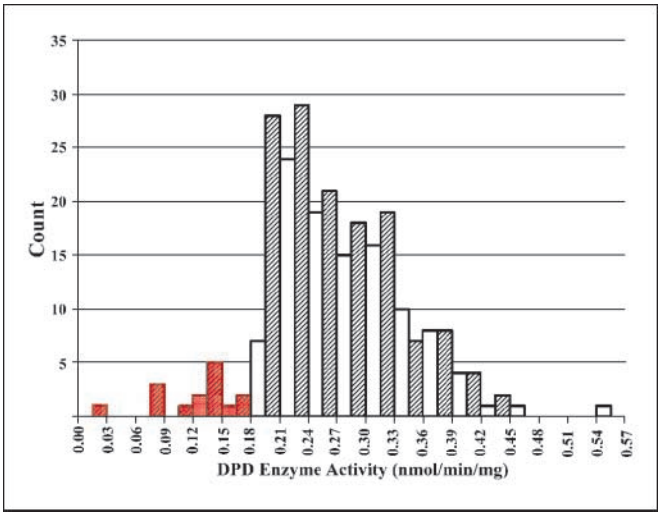

Fig. 1. Distribution of PBMC DPD enzyme activity in healthy African-American and Caucasian volunteers. Distributions of PBMC DPD enzyme activity from healthy African-American (n = 149; *hatched columns*) and Caucasian subjects (n = 109; *clear columns*). For reference, individuals with DPD deficiency are shown (PBMC DPD enzyme activity ≤0.18 nmol/min/mg protein; *red columns*). African-Americans were found to have significantly lower PBMC DPD enzyme activity than Caucasians (P = 0.002). Twelve African-Americans were DPD-deficient compared with three Caucasians. The prevalence of DPD deficiency among the African-American and Caucasian populations was 8.0% and 2.8%, respectively.

UraBT PDR_{180} and DOB_{50} distributions in the study population. The PDR_{180} values and UraBT DOB_{50} concentrations from the entire study population (n = 258) were both normally distributed. The mean (±SE) PDR_{180} value observed from the entire study population was 53.1 ± 0.5%. The mean (±SE) DOB_{50} concentration observed from the entire study population (n = 258) was 174.3 ± 2.1 DOB. Based on the previously established UraBT DOB_{50} cut-point, 19 volunteers (7.4%) were classified as DPD-deficient.

Characterization of UraBT PDR_{180} and DOB_{50} values in healthy African-American and Caucasian volunteers. PDR_{180} values from African-American (n = 149) and Caucasian volunteers (n = 109) were both normally distributed. The distribution of PDR_{180} values in African-Americans and Caucasians were both positively skewed (with the tail extending toward higher PDR_{180} values). The coefficient of skewness was 0.74 and 0.45, respectively.

African-American volunteers also showed significantly lower PDR_{180} values compared with Caucasian volunteers (P = 0.03). The mean (±SE) PDR_{180} value observed in African-Americans and Caucasians was 52.3 ± 0.6% and 54.3 ± 0.7%, respectively.

The DOB_{50} distributions observed from African-American (n = 149) and Caucasian (n = 109) volunteers were normally distributed (Fig. 2). The DOB_{50} distribution in African-Americans was negatively skewed (with the tail extending into the low range of $^{13}CO_2$ breath concentrations), whereas the distribution in Caucasian volunteers was positively skewed (with the tail extending toward higher $^{13}CO_2$ breath concentrations).The coefficient of skewness was −0.23 and 0.19, respectively.

African-Americans also showed a significantly lower DOB_{50} concentrations compared with Caucasians (P = 0.004). The mean (±SE) DOB_{50} observed in African-Americans and Caucasians was 169.1 ± 2.9 and 181.4 ± 3.0 DOB, respectively. Of the 19 volunteers who screened positive for DPD deficiency by UraBT, 16 were African-American and 3 were Caucasian.

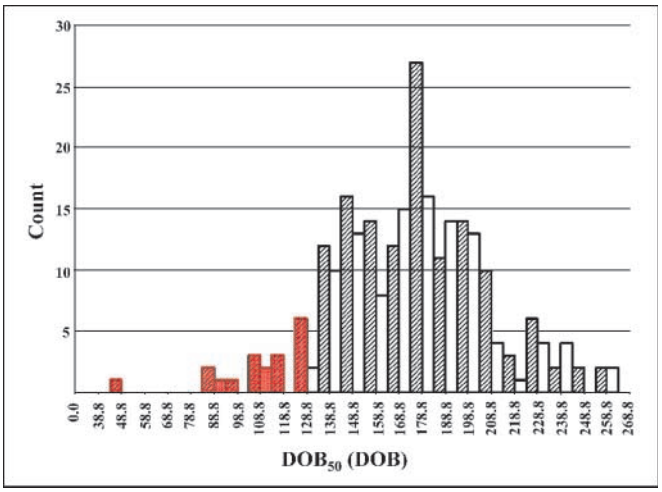

Fig. 2. Rapid detection of DPD deficiency in healthy African-American and Caucasian volunteers. UraBT DOB_{50} distributions from healthy African-American volunteers (n = 149; *hatched columns*) and Caucasian volunteers (n = 109; *clear columns*). For reference, individuals who screened positive for DPD deficiency are shown (UraBT DOB_{50} < 128.9 DOB; *red columns*). African-Americans were found to have significantly lower UraBT DOB_{50} values than Caucasians (P = 0.004). Based on the UraBT DOB_{50} cut-point, 19 participants (7.4% of the study population) screened positive for DPD deficiency.

Discussion

DPD deficiency predisposes patients with cancer to severe, life-threatening 5-FU toxicity. Recently, we developed and optimized the UraBT to rapidly (<1 hour) screen cancer patients for reduced DPD enzyme activity (18). Subsequently, we did a pharmacokinetic validation of the UraBT by characterizing relationships present among UraBT-associated breath $^{13}CO_2$ metabolite formation, plasma [2-^{13}C]dihydrouracil formation, plasma [2-^{13}C]uracil clearance, and PBMC DPD enzyme activity in normal and DPD-deficient subjects (19). More recently, we showed that the UraBT is a rapid method suitable for population studies by screening 13 East Indian subjects for DPD deficiency subsequent to our initial identification and characterization of DPD deficiency in an East Indian cancer patient with severe 5-FU toxicity (1). In the current study, we used the UraBT to screen for DPD deficiency in a population composed of 258 Caucasian and African-American volunteers.

Racial differences, resulting from genetic variability, have been observed in the activity of several drug-metabolizing enzymes such as cytochrome P450 2C19 (CYP 2C19), *N*-acetyltransferase, and thiopurine methyltransferase (29–31). In the current study, we showed that racial differences are present in DPD enzyme activity. Specifically, we observed significantly lower PBMC DPD enzyme activity in the African-American population compared with the Caucasian population. Others have also observed racial differences in DPD enzyme activity. Sohn et al. observed increased PBMC DPD enzyme activity in Koreans (n = 114) compared with the activities that had previously been reported in Caucasians (32). Comparatively, a large population study of 34,200 Japanese infants did not detect DPD deficiency in any of the enrolled subjects (33).

Our observation of significantly lower PBMC DPD enzyme activity in African-American volunteers suggests that African-Americans have significantly reduced *in vivo* pyrimidine catabolism compared with Caucasians. Recently, we did a pharmacokinetic evaluation of the UraBT and showed that the UraBT DOB_{50} is significantly related to markers of [2-^{13}C]uracil degradation (i.e., clearance, half-life, and area under the curve) and [2-^{13}C]dihydrouracil formation (C_{max}, T_{max}, and rate of appearance; ref. 19). In the current study, we observed that African-Americans had significantly lower UraBT DOB_{50} values compared with Caucasians. Furthermore, African-Americans metabolized a significantly lower percentage dose of [2-^{13}C]uracil to $^{13}CO_2$ compared with Caucasians. Taken together, these results suggest that African-Americans have a significantly lower *in vivo* pyrimidine catabolism compared with Caucasians, which may put them at risk for increased DPD-mediated 5-FU toxicity.

A recent study of African-American and Caucasian patients with colorectal cancer examined whether racial differences in 5-FU toxicity were present (20). African-American patients showed significantly increased leukopenia and anemia compared to Caucasian patients with colorectal cancer (20). Unfortunately, the DPD enzyme activity of the two patient populations was not measured. Additional research examining racial differences in DPD enzyme activity and the occurrence of DPD-mediated 5-FU toxicity in cancer patients are warranted.

Earlier clinical studies observed increased 5-FU toxicity in women compared with men (34, 35). This led to the hypothesis that women may have lower DPD enzyme activity compared with men. However, subsequent studies were unable to prove or disprove this hypothesis (15, 16, 36–38). In the current study, we observed significantly lower DPD enzyme activity in women compared with men. Furthermore, stratification by gender and race showed that African-American women had the lowest DPD enzyme activity of any other race-gender group ($P \leq 0.003$ for all pairwise comparisons). These results suggest that African-American women, in particular, may be at increased risk of DPD-mediated 5-FU toxicity. This is of interest as the *DPYD* gene is located on chromosome 1p22 and has been previously described as having an autosomal codominant inheritance pattern (39, 40).

Recent clinical studies have observed reduced activity in other enzymes of the pyrimidine catabolic pathway. In particular, reduced dihydropyrimidinase and β-ureidopropionase activities have been observed in a 5-FU toxic patient and children with neurologic abnormalities (41–44). It may be possible to detect these deficiencies in pyrimidine catabolism using the UraBT. In order for $^{13}CO_2$ to be released in the breath, the [2-^{13}C]uracil substrate must be catabolized by DPD, dihydropyrimidinase, and β-ureidopropionase. Individuals with reduced dihydropyrimidinase and β-ureidopropionase activities would be expected to have decreased $^{13}CO_2$ breath concentrations compared with individuals with normal pyrimidine catabolisms. In the current study, we observed reduced UraBT DOB_{50} concentrations in four subjects with normal DPD enzyme activity. Examination of the entire breath $^{13}CO_2$ concentration-time profiles from these four subjects showed that two of the four subjects had breath profiles with an early T_{max} (30 and 40 minutes; data not shown) and a C_{max} above the "cut-point" (142.8 and 139.2 DOB; data not shown). These data suggest that normal pyrimidine catabolism is present in these two subjects. However, two of the four subjects showed reduced breath $^{13}CO_2$ concentration-time profiles compared with normal subjects (data not shown). These data suggest that

altered pyrimidine catabolism may be present. Genotypic evaluation of the *DPYS* and *BUP* genes from these two subjects are currently being conducted by our laboratory.

In summary, we applied the UraBT to screen for DPD deficiency in a population of African-Americans and Caucasians. We showed that the African-American population, particularly African-American women, had an increased prevalence of DPD deficiency and significantly reduced PBMC DPD enzyme activity and [2-^{13}C]uracil catabolism. These results suggest that African-Americans may be at risk for 5-FU toxicity resulting from reduced catabolism. Currently, genotypic studies are being done by our laboratory to examine the *DPYD* gene of all volunteers with reduced DPD enzyme activity to identify the molecular basis of DPD deficiency. Future studies will prospectively apply the UraBT to identify cancer patients at risk of developing 5-FU toxicity due to reduced DPD enzyme activity.

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