An odyssey can be defined as “a long wandering and eventful journey” (1). Traveling together with many collaborators, from a National Cancer Institute–sponsored phase I trial to drug metabolism to genomics, we learned, over more than a decade, the essence of pharmacogenetics, a field with which we had only modest familiarity before this journey. The story began with the suggestion of the National Cancer Institute in the early 1990s that The University of Chicago conduct a new phase I study of irinotecan on the weekly schedule, with carte blanche to incorporate colony-stimulating factors and antidiarrheals. From a clinical perspective, the incremental knowledge was modest, as the maximally tolerated dose (if one used an aggressive definition of “tolerated”) was 145 mg/m² weekly (4 of 6 weeks), with or without the use of filgastrim or additional antidiarrheals (2). It was that clinical experience, however, that allowed us to identify SN-38 glucuronide as the major metabolite of irinotecan in humans, and to suggest that the extent of glucuronidation was inversely correlated with irinotecan’s dose-limiting gastrointestinal toxicity on the weekly schedule (3).

In that seminal article (3), we suggested that the variability in glucuronidation may be genetic and that it may be beneficial to induce glucuronidation. These suggestions (as well as a proposal to inhibit biliary excretion of SN-38) were also encapsulated as claims in a provisional patent filed through ARCH Development Corporation, the default assignee in 1994 for all inventions by faculty at The University of Chicago. (We also confirmed our initial clinical findings in a separate cohort of patients; ref. 4).

With the provisional patent filed (subsequently converted to a full patent), we immediately began to try to work with Upjohn (which had the U.S. rights to irinotecan) to develop our ideas with the goal of confirming our initial hypotheses and translating our patent claims into clinical practice. Unfortunately, although some of the Upjohn scientists were interested in our results, there was never any apparent major interest at Upjohn (or its descendants Pharmacia and Pfizer) in our work until the Food and Drug Administration (FDA) became interested in 2004, a decade later.

Thus, we proceeded with our own development of the dual strategies we proposed: (a) identification of individuals with decreased glucuronidation of SN-38 and (b) modulation of the pharmacokinetics of irinotecan. The latter strategy was of keen interest to us because of the potential of this approach to have an impact on the therapeutic index of this exciting drug. We explored the use (initially in rats) of cyclosporine A to inhibit biliary excretion and of phenobarbital to induce glucuronidation (4, 5). (We eventually conducted both phase I and phase II trials of this approach, which remains to be of some interest; refs. 6, 7).

Given the lack of pharmaceutical company interest in our modulation approach, we turned our focus to understanding the basis for variability in glucuronidation. These studies were facilitated by our access to human liver tissue from patients with Crigler-Najjar syndrome, a genetic deficiency of bilirubin glucuronidation. We fortuitously identified that these livers were also unable to glucuronidate SN-38 and confirmed that UGT1A1 (which had recently been cloned) was able to do so (8). This result was particularly exciting as a common variant in the UGT1A1 gene had recently been identified, which was associated with variability in bilirubin conjugation (glucuronidation) and Gilbert’s syndrome (9, 10). Thus, we not only had a candidate gene, we had a candidate polymorphism.

This polymorphism, now known as UGT1A1*28, is an extra TA repeat (7 versus 6) in the UGT1A1 promoter. The UGT1 locus is complex, consisting of one gene with multiple first exons and four common exons (11). The four common exons are denoted as exons 2 to 5 and each of the first exons is denoted by the UGT1 isoform for which it specifically encodes. Although they are loosely numbered from 1 to 13 in a 3’ to 5’ direction (e.g., UGT1A1 is the most 3’), they do not consistently follow that order.

While this work was proceeding, we learned that our original patent had been divided into sets of therapeutic (i.e., modulation of pharmacokinetics) and diagnostic (i.e., phenotype or genotype tests to predict glucuronidation) claims. The consequence of this division was that only one of the divided sets of claims could be prosecuted at a time. With our input, ARCH Development elected to prosecute the therapeutic claims.

Given that we had a candidate polymorphism and an inventory of human liver tissue, we proceeded to conduct an in vitro pharmacogenetic study. We isolated DNA for genotyping and made microsomes for phenotyping of SN-38 glucuronidation. These results (using 44 liver specimens) were supportive of our hypothesis that genetic variability in the UGT1A1 promoter was associated with SN-38 glucuronidation and irinotecan toxicity (12).
Meanwhile, we received some good news and bad news. The good news was that our patent had been issued (13). The bad news was that ARCH Development had failed to make a decision to prosecute the diagnostic claims before the issuance of this patent in 1998, leading to loss of the original claims. Furthermore, under U.S. law, they would now be considered prior art for all future patents filed by us.

As an additional complication, there was increasing preference among U.S. clinicians for the European schedule, which was a single dose every 3 weeks. Therefore, we made the decision to use this schedule for our prospective trial. As the FDA had not yet approved this schedule, however, there was no clarity about the dose and we (wrongly) selected 300 mg/m\(^2\). After approval by the FDA of a dose of 350 mg/m\(^2\) on this schedule, we analyzed the data on our 20 patients treated and amended the protocol to the higher dose. Fortuitously, there was a significant relationship between UGT1A1 genotype and the neutrophil nadir (Fig. 1; ref. 14). (Of note, we had not recognized the marked schedule dependency of the gastrointestinal toxicity and had actually planned to analyze the relationship of genotype to diarrhea as the primary end point, but we observed minimal diarrhea at this dose and schedule).

We then enrolled an additional 60 evaluable patients, the prospectively determined sample size required to test our hypothesis about diarrhea. Even at the higher dose, there was much less diarrhea than we anticipated. Thus, we analyzed the relationship of genotype to neutropenia, finding a striking relationship as hypothesized (15). Patients homozygous for UGT1A1*28 (7/7) had a 50% incidence of grade 4 neutropenia whereas the noncarriers of this polymorphism (6/6) had a 0% incidence of grade 4 neutropenia. Patients heterozygous for the polymorphism (6/7) had an intermediate phenotype. Similar findings were observed in analyzing the relationship of genotype to SN-38 AUC or the extent of glucuronidation.

These findings came to the attention of the FDA Office of Clinical Pharmacology and Biopharmaceutics, leading to a meeting on November 3, 2004 of the FDA Advisory Committee on Pharmaceutical Science, where the data of our group and those of others conducting similar studies around the world were summarized. Based on these results, the Advisory Committee voted unanimously to recommend labeling changes to reflect UGT1A1*28 genotype as a risk factor for the hematologic toxicity of irinotecan.

Pfizer was also invited to this FDA meeting to provide their perspective (before the vote). They voiced their commitment to safety and the application of pharmacogenetics to “getting important information during the whole drug development process.”

Approximately 7 months after the meeting, the FDA approved a label revision reflecting the Advisory Committee recommendations. The medical oncology community has been slowly educated about this label revision, which includes a warning that patients homozygous for UGT1A1*28 have an increased risk of toxicity and should receive a lower starting dose.

Recently, the Mayo Clinic has launched a commercial diagnostic test for this polymorphism after completing a licensing arrangement with The University of Chicago. (ARCH Development no longer exists.) A sublicense has been executed between the Mayo Clinic and Third Wave Technologies, which had announced in August 2005 the FDA approval of its diagnostic test. Genzyme has announced a relationship with Third Wave and that it is the “preferred laboratory partner for marketing UGT1A1 in the United States.” Thus, 2006 now seems to be the year in which our technology to predict irinotecan toxicity may finally be translated into clinical practice.

There are still significant barriers to the usage of this test, however. The first barrier is education of the clinician about the potential value (and limitations) of testing. The second barrier is potential limitations on reimbursement. Although the test will cost much less than the current cost of a single dose of irinotecan, its reimbursement pattern is unknown, as the test has recently been brought to market. In addition, many physicians and patients remain leary of genetic testing because of the potential costs to both patient and family members of ascertaining genetic information (16). Although this is a test for a risk of toxicity to a drug, UGT1A1 polymorphisms have been associated with a number of malignant and nonmalignant conditions (17–19).

This is only a wayside in the odyssey, as many unanswered questions remain. Focusing only on the promoter

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Fig. 1. Metabolism of irinotecan. Irinotecan (CPT-11) is activated by carboxylesterase 2 (CES2) to SN-38, which is conjugated (a detoxification step) by UGT1A1 to SN-38 glucuronide (SN-38G). The promoter polymorphism in UGT1A1 affects the rate of this reaction. In addition, irinotecan is oxidized (detoxification) to 1-ethyl-10-(4-N-(5)-aminopentanoic acid-1-piperidino)carbonyloxycamptothecin (APC).
polymorphism, what is the best dose for each of the three major genotypes (6/6, 6/7, 7/7)? What do we do with patients with minor genotypes (e.g., 5 or 8 repeats) (20, 21)? Assuming a higher than standard dose can be safely administered to a subset of patients (e.g., 6/6), is that more efficacious? Does genotype associate with resistance or susceptibility? Is the causative polymorphism in the promoter, or is it another polymorphism in linkage disequilibrium (e.g., −3156 G/A) (15)? Are there relevant polymorphisms in other first exons of UGT1 that affect the function or expression of other isoforms that glucuronidate SN-38 (e.g., UGT1A7, UGT1A9, and UGT1A10), affecting pharmacokinetics, gastrointestinal toxicity, or resistance? Are there relevant polymorphisms in other genes that affect either the pharmacokinetics or pharmacodynamics of irinotecan?

For those wishing to join this odyssey, be forewarned, especially those “travelers” in Asia, where the genetics of UGT1A1 are quite different (22, 23). For those who have embarked on their own odysseys involving translational research, be prepared to recruit experts in clinical trials, laboratory science, patent law, and technology transfer, as well as mastering these areas yourself. Work hard, fasten your seat belt, and travel safely.

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References
