YAFFE AWARD LECTURE

Pharmacogenomics of Adverse Effects of Anti-Leukemic Agents in Children

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I am honored to receive the 2011 Yaffe Award from the Pediatric Pharmacy Advocacy Group. Dr. Yaffe's contributions to pediatric clinical pharmacology were enormous. In our own research, we have sought to improve the effectiveness of therapy for children with cancer through our research and by constantly working to translate research findings into the clinic.

Pharmacogenetics of childhood acute lymphoblastic leukemia (ALL) includes determining the inherited basis for important drug-induced phenotypes in this disease. Phenotypes include efficacy phenotypes such as *ex vivo* drug sensitivity, minimal residual disease, and the risk of relapse. The disease also includes toxicity phenotypes such as thiopurine-induced myelosuppression, asparaginase-induced allergy, and glucocorticoid-induced osteonecrosis or methotrexate-induced gastrointestinal toxicity. I will review several examples of important pharmacogenetic relationships that have been elucidated in children with ALL.

The approaches that we have taken to elucidate pharmacogenetic relationships in ALL include candidate gene approaches and genome-wide approaches. The first example of a candidate gene approach involves the gene for the enzyme thiopurine methyltransferase (TPMT). We have shown that polymorphisms in thiopurine methyltransferase affect mercaptopurine acute toxicity, mercaptopurine chronic toxicity, and the probability of cure.[1–7] At St. Jude, we have been individualizing the dose of thiopurines based on the inherited genotype for TPMT since the early 1990s. For the 1 in 400 individuals who is homozygous for variant TPMT alleles, the thiopurine is decreased dramatically, with over a 10-fold decrease in the daily dose compared to patients with normal TPMT activity. The 10% of the population who are heterozygous, carrying one wild-type and one variant allele, normally require somewhat reduced doses of thiopurines. The 90% of patients who are homozygous for wild-type TPMT alleles are usually able to tolerate normal doses of the drug. Individuals who are homozygous for variant alleles are at high risk of life-threatening myelosuppression if they are treated with normal doses of the drug, whereas those patients who are homozygous for wild-type alleles tend to be at a lower risk of acute myelosuppression but also at a slightly increased risk of relapse.[8]

The proportion of patients with ALL who can avoid a dose decrease of 6-mercaptopurine is based on their TPMT status, that is, patients who carry the wild-type alleles are more able to avoid a dose decrease (Figure 1) than those who are heterozygotes or homozygous-bearing individuals.[6] These findings were closely mimicked by a murine model of TPMT status in which the knockout mice were unable to tolerate full doses of 6-mercaptopurine without experiencing fatal toxicity.[9]

Using this knowledge at St. Jude, we began to adjust the doses of mercaptopurine in patients based on a combination of their TPMT genotype and their clinical tolerance. After we introduced genotype-adjusted dosing for thiopurines, we observed no change in the cumulative incidence of acute adverse effects in individuals who were carrying variant alleles for TPMT compared to that of those who were wild-type for TPMT,[10] consistent with the notion that adjusting doses of thiopurines could attenuate or abrogate the adverse effect of thiopurines that is attributable to TPMT status. We also showed that this approach to adjusting thiopurine doses based on TPMT genotype and acute myelosuppression did not result in any increased risk of relapse in the patients who

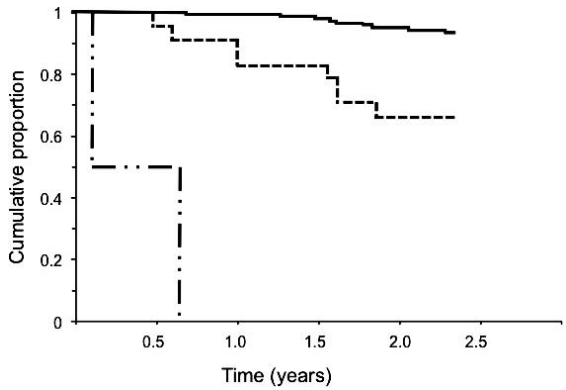

Figure 1. The proportion of patients with ALL avoiding a dose decrease of 6-mercaptopurine. ALL, acute lymphoblastic leukemia. Solid line represents patients who are wild-type for thiopurine methyltransferase (TPMT); dashed line represents those who are heterozygous for TPMT; dashed and dotted line represents those few patients who are homozygous deficient for TPMT.

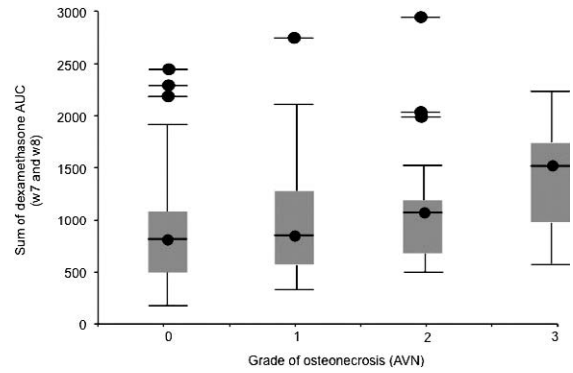

Figure 2. Higher dexamethasone plasma AUC was associated with higher grades of osteonecrosis.[18] The sum of dexamethasone AUC (area under the concentration time curve) is the sum of measures of AUC taken at week 7 and week 8 of reinduction therapy (w7 and w8)

were heterozygous for TPMT gene status.[11] This led us to be advocates for assessing TPMT status as part of therapeutic drug monitoring for thiopurines.

Ultimately, the work performed at St. Jude and around the world indicating that thiopurine doses would be better tolerated without compromising desired efficacy has led to the development of therapeutic guidelines for adjusting thiopurine dosages based on TPMT status.[12] These Clinical Pharmacogenetic Implementation Consortium guidelines now exist not only for thiopurines and TPMT but also for warfarin,[13] codeine and cytochrome P450 (CYP) 2D6,[14] and clopidogrel and CYP2C19.[15]

One of the dose-limiting adverse effects of ALL therapy is glucocorticoid-induced osteonecrosis.[16] We observed substantial interpatient variability in dexamethasone pharmacokinetics among children with ALL, such that systemic exposure was approximately twofold higher in teenagers than in toddlers, based on the lower clearance observed in adolescents.[17] This is consistent with a higher frequency of osteonecrosis in teenagers than in younger children with ALL.

In a prospective study in which we characterized dexamethasone pharmacokinetic clinical risk factors and genetic risk factors for osteonecrosis, we observed that the plasma area under the curve for dexamethasone was related to the risk of osteonecrosis (Figure 2) with a higher grade of osteonecrosis related to a higher area under the concentration time curve (AUC) for dexamethasone.[18] We have also observed the tendency for a higher frequency of osteonecrosis in those patients who are exposed to higher levels of asparaginase. This was recapitulated in a murine model (Figure 3) for dexamethasone-induced necrosis in which the frequency of osteonecrosis was higher in mice treated with a combination of dexamethasone plus asparaginase than it was in those treated with dexamethasone alone.[19]

In St. Jude Protocol Total XV, we comprehensively analyzed risk factors for osteonecrosis.[18] We found that both age >10 years and treatment on the standard high-risk rather than the low-risk arm of the treatment protocol were associated with osteonecrosis, whereas race and gender were not. There was also a tendency for those with osteonecrosis to have lower serum albumin and higher serum cholesterol as well as higher dexamethasone plasma AUC. In this context, after performing a genome-wide association analysis, we observed a significant association between single nucleotide polymorphisms (SNP) in the acid phosphatase 1 (ACP1) locus and the risk of osteonecrosis in these patients.

One of the agents most commonly used to treat childhood ALL is methotrexate.[20] We and others have shown that the risk of relapse is greatest in patients who have high clearance of high-dose methotrexate;[21] in other words, those who have low plasma concentrations of methotrexate are at a higher risk of relapse. Variation in the plasma concentration of methotrexate may be due to intraindividual variations in methotrexate clearance. Methotrexate clearance varies substantially among patients who have apparently normal renal function, such that even patients receiving exactly the same dose could easily vary fourfold in their

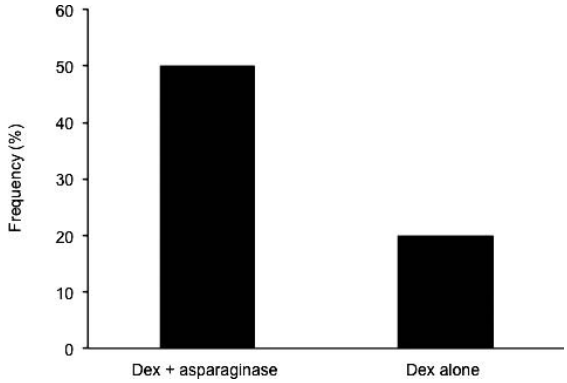

Figure 3. Asparaginase increases frequency and hastens onset of dexamethasone osteonecrosis in a murine model.[19]

systemic exposure or plasma AUC to methotrexate (Figure 4).

Because exposure to methotrexate has been related not only to relapse but also to acute toxicity,[22–24] we performed a genome-wide analysis to identify genetic variations associated with methotrexate clearance variability. We observed that there were several SNPs in a single gene, called *SLC01B1*, that were associated with variation in methotrexate clearance.[25] This variation in methotrexate clearance was also related to variation in the risk of gastrointestinal toxicity.

We went on to sequence the *SLC01B1* gene in a cohort of 699 children with leukemia to determine what the relative importance of rare versus common SLC01B1 variants are to interindividual variability in clearance of methotrexate.[26] We found 69 single nucleotide polymorphisms that had not been interrogated on the genome-wide array. Approximately two-thirds of them had never been previously described, and several of them were nonsynonymous. We applied *in silico* functional algorithms to predict which of these polymorphisms were damaging versus which were tolerated, and we observed that, although there was not any statistically significant overrepresentation of any nonsynonymous SNPs among patients with low medium versus high clearance, we did observe that there was an overrepresentation of damaging nonsynonymous SNPs in patients who had low methotrexate clearance compared to those who had the highest clearance. Combining all information for common and rare variants, we found that rare nonsynonymous SNPs in this single gene explained approximately 18% of the population variability in methotrexate clearance that could be attributable to *SLC01B1*.

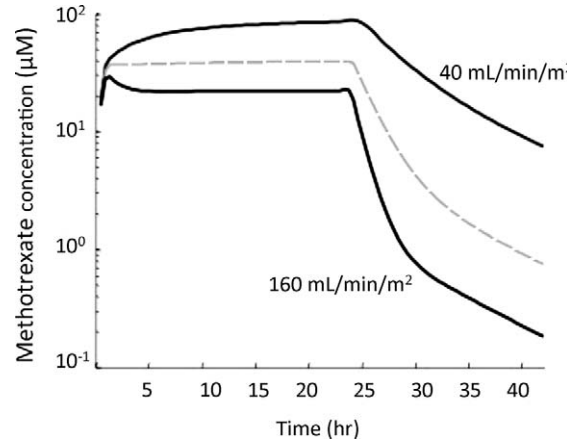

Figure 4. Interindividual variability in methotrexate clearance substantially affects drug exposure (fourfold range), as illustrated for 3 representative concentrations versus time plots for patients with low, medium, and high clearance receiving 5 g/m^2 doses.

Using a combination of candidate gene approaches and genome-wide approaches, we and others are characterizing the inherited genomic variability that is important for childhood ALL. However, throughout the last 30 years of research, multiple pharmacogenetic polymorphisms have been well characterized and have strong data indicating that such polymorphisms should be used in making prescribing decisions.

At St. Jude, we have taken on a new project to overcome barriers to integration of pharmacogenetic tests into clinical care through a clinical treatment protocol called *Pharmacogenetics for Kids* (PG4KDS), by which we will introduce preemptive pharmacogenetics into the patient's medical record. The goal of this study is to migrate pharmacogenetic tests from the laboratory into routine patient care so that the genotypes are available preemptively. Using an extensive system of computational decision support, rules are built by which alerts will fire for clinicians who order a high-risk medication for a patient with a high-risk genotype. The process (Figure 5) behind the PG4KDS protocol is that a patient is enrolled in the study, they provide a blood sample, and their DNA is genotyped using an array-based technology in a Clinical Laboratory Improvement Amendments-certified laboratory environment. Each of the genotypes encompassed in the array is classified as to its clinically eligible genotype, that is, the ability, in principle, to be moved from the laboratory into the medical record. The currently used array (DMET Plus; Affymetrix, Santa Clara, CA) interrogates 225 genes; currently the plan is for most of

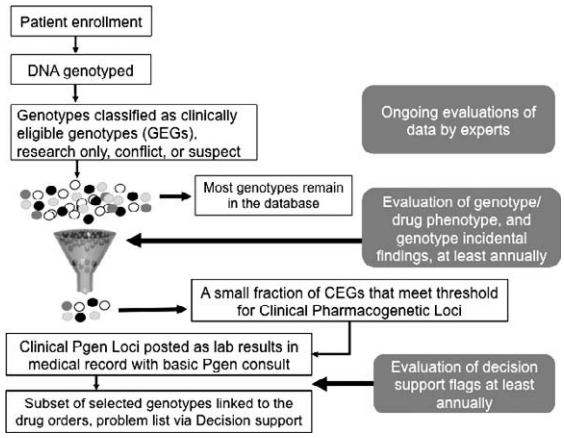

Figure 5. Process for integrating pharmacogenetics test results into the medical record as part of St. Jude's PG4KDS Research Protocol. CEGs, clinically eligible genotypes; Pgen, pharmacogenomics.

those genes to have their data remain in the research database. However, using a process characterized by ongoing evaluation of existing data by experts[27] and evaluation of genotype/phenotype relationships, the PG4KDS team prioritizes a small fraction of these clinically eligible genotypes that meet the threshold for moving over the firewall from the laboratory into the clinical medical record. Recommendations are approved by the Pharmacy and Therapeutics Committee. These clinical pharmacogenetic loci have genotypes that are then posted into the laboratory results in the medical record, and each diplotype for each gene is linked with a genotype/diplotype-specific pharmacogenetic consult. The genotype result and the consult will be posted for each eligible patient for each eligible gene, and it is anticipated that the number of genes moved from the research databases into the clinical electronic medical record will slowly increase over time. For that small subset of selected high-priority genotypes, such as the TPMT heterozygote patient or poor metabolizer of CYP2D6, a problem list entry is generated automatically based on the high-risk genotype. The pharmacy team, working with the informatics team at St. Jude, has built decision-supported alerts that will fire if a clinician writes an order for a high-risk drug to be delivered to a patient with an underlying high-risk genotype.[28] Because the genotypes will have been determined preemptively, these results will be present in the patient's medical record and available for future use. In fact, a principle of the PG4KDS protocol is that genotypes are lifetime results, and we are working to obtain consent and to set up systems whereby these genotypes are given to the patients while they are children, but, of course, these results will continue to be applicable as the child ages and will be useful for other clinicians in the future.

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ABBREVIATIONS ALL, acute lymphoblastic leukemia; AUC, area under the concentration time curve; CYP, cytochrome P450; 6MP, 6-mercaptopurine; Pharmacogenetics for Kids, PG4KDS; SNP, single nucleotide polymorphisms; TPMT, thiopurine methyltransferase

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REFERENCES

1. Evans WE, Hon YY, Bomgaars L, et al. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J Clin Oncol*. 2001;19(8):2293–2301.
2. Krynetski EY, Schuetz JD, Galpin AJ, et al. A single point mutation leading to loss of catalytic activity in human thiopurine S-methyltransferase. *Proc Natl Acad Sci U S A*. 1995;92(4):949–953.
3. Yates CR, Krynetski EY, Loennechen T, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. *Ann Intern Med*. 1997;126(8):608–614.
4. Relling MV, Yanishevski Y, Nemec J, et al. Etoposide and antimetabolite pharmacology in patients who develop secondary acute myeloid leukemia. *Leukemia*. 1998;12(3):346–352.
5. Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. *Blood*. 1999;93(9):2817–2823.

6. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J Natl Cancer Inst*. 1999;91(23): 2001–2008.
7. Relling MV, Rubnitz JE, Rivera GK, et al. High incidence of secondary brain tumours after radiotherapy and antimetabolites. *Lancet*. 1999;354(9172):34–39.
8. Relling MV, Dervieux T. Pharmacogenetics and cancer therapy. *Nat Rev Cancer*. 2001;1(2):99–108.
9. Hartford C, Vasquez E, Schwab M, et al. Differential effects of targeted disruption of thiopurine methyltransferase on mercaptopurine and thioguanine pharmacodynamics. *Cancer Res*. 2007;67(10):4965–4972.
10. Stocco G, Cheok MH, Crews KR, et al. Genetic polymorphism of inosine triphosphate pyrophosphatase is a determinant of mercaptopurine metabolism and toxicity during treatment for acute lymphoblastic leukemia. *Clin Pharmacol Ther*. 2009;85(2):164–172.
11. Relling MV, Pui CH, Cheng C, Evans WE. Thiopurine methyltransferase in acute lymphoblastic leukemia. *Blood*. 2006;107(2):843–844.
12. Relling MV, Gardner EE, Sandborn WJ, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. *Clin Pharmacol Ther*. 2011;89(3):387–391.
13. Johnson JA, Gong L, Whirl-Carrillo M, et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clin Pharmacol Ther*. 2011;90(4):625–629.
14. Crews KR, Gaedigk A, Dunnenberger HM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for codeine therapy in the context of cytochrome P450 2D6 (CYP2D6) genotype. *Clin Pharmacol Ther*. 2012;91(2):321–326.
15. Scott SA, Sangkuhl K, Gardner EE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. *Clin Pharmacol Ther*. 2011;90(2):328–332.
16. Relling MV, Yang W, Das S, et al. Pharmacogenetic risk factors for osteonecrosis of the hip among children with leukemia. *J Clin Oncol*. 2004;22(19):3930–3936.
17. Yang L, Panetta JC, Cai X, et al. Asparaginase may influence dexamethasone pharmacokinetics in acute lymphoblastic leukemia. *J Clin Oncol*. 2008;26(12):1932–1939.
18. Kawedia JD, Kaste SC, Pei D, et al. Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. *Blood*. 2011; 117(8):2340–2347; quiz 2556.
19. Yang L, Boyd K, Kaste SC, et al. A mouse model for glucocorticoid-induced osteonecrosis: effect of a steroid holiday. *J Orthop Res*. 2009; 27(2):169–175.
20. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med*. 2004; 350(15):1535–1548.
21. Evans WE, Relling MV, Rodman JH, et al. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *N Engl J Med*. 1998;338(8):499–505.
22. Relling MV, Fairclough D, Ayers D, et al. Patient characteristics associated with high-risk methotrexate concentrations and toxicity. *J Clin Oncol*. 1994;12(8):1667–1672.
23. Pauley JL, Panetta JC, Schmidt J, et al. Late-onset delayed excretion of methotrexate. *Cancer Chemother Pharmacol*. 2004;54(2):146–152.
24. Garre ML, Relling MV, Kalwinsky D, et al. Pharmacokinetics and toxicity of methotrexate in children with Down syndrome and acute lymphocytic leukemia. *J Pediatr*. 1987;111(4): 606–612.
25. Trevino LR, Shimasaki N, Yang W, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol*. 2009;27(35):5972–5978.
26. Ramsey LB, Bruun GH, Yang W, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Res*. 2012;22(1):1–8.
27. Relling MV, Klein TE. CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clin Pharmacol Ther*. 2011;89(3):464–467.
28. Crews KR, Cross SJ, McCormick JN, et al. Development and implementation of a pharmacist-managed clinical pharmacogenetics service. *Am J Health Syst Pharm*. 2011;68(2):143–150.