Integration of Germline Pharmacogenetics Into a Tumor Sequencing Program

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Abstract

Purpose—Evidence-based guidelines inform treatment decisions for patients for whom germline genetic information is available. Our real-time tumor sequencing program, which makes precision treatment decisions for patients with cancer, produces matched germline information, providing a unique opportunity to efficiently implement pharmacogenetics and benefit patients.

Methods—The germline genetic database from the Michigan Oncology Sequencing (MI-Oncoseq) program was searched for 21 clinically actionable polymorphisms in five cancer-relevant genes: *TPMT*, *DPYD*, *CYP2C19*, *CYP3A5*, and *UGT1A1*. Residual germ line DNA was sent to an external Clinical Laboratory Improvement Amendments–approved laboratory for confirmatory genotyping. The medical records of MI-Oncoseq patients with actionable phenotypes were searched for receipt of relevant drugs and to determine whether having genetic information at the time of treatment would have led to a treatment recommendation.

Results—All nine variants in *TPMT*, *DPYD*, and *CYP2C19* that were detected in MI-Oncoseq were confirmed by external genotyping. Genotype determinations could not be made for *CYP3A5*3*, *UGT1A1*28*, or *UGT1A1*80*. On the basis of retrospective assessment of 115 adult and pediatric patient records, 4.3% (n = 5) had a potentially clinically actionable phenotype for *TPMT*, *DPYD*, or *CYP2C19* and received a relevant medication. After accounting for differences in adult and pediatric recommendations, three of these patients could have received a treatment recommendation at the time of prescribing.

Conclusion—Germline genotype determinations for *TPMT*, *DPYD*, and *CYP2C19* can be used to make evidence-based treatment recommendations in MI-Oncoseq patients. Although the proportion of patients for whom recommendations can be made is small, this added value to MI-Oncoseq and patient care comes at no additional genotyping cost. Pharmacogenetic assessment should be integrated into tumor sequencing programs that genotype matched germline DNA; however, the complexity and additional cost of implementing pharmacogenetics remain challenging.

INTRODUCTION

The genomes of patients (germline) and their tumors (somatic) each provide useful information to guide precision medicine in oncology. Validated pharmacogenetic associations exist for several germline polymorphisms with commonly used cancer treatments, including thiopurines (*TPMT*),[1] fluorouracil (FU)/capecitabine (*DPYD*),[2] irinotecan (*UGT1A1*),[3] and tacrolimus (*CYP3A5*).[4] Additionally, there are several known associations with supportive care agents commonly used in patients during treatment, such as fungal prophylaxis with voriconazole (*CYP2C19*)[5] and antiemetic treatment with ondansetron (*CYP2D6*).[6]

Despite their established clinical validity, few of these associations have been implemented into clinical practice. St Jude Children's Research Hospital has led the way, implementing

preemptive pharmacogenetic testing to guide personalized treatment of several gene-drug pairs.[7] The experiences of early adopters has identified formidable challenges to implementing pharmacogenetics into clinical practice,[8,9] which require substantial investment and expertise.[10,11]

The primary bottleneck in the implementation of pharmacogenetics is a lack of evidence that pharmacogenetic testing meaningfully improves patient outcomes. Although there is debate about the necessity and feasibility of demonstrating clinical utility for pharmacogenetic implementation,[12,13] the lack of uptake indicates that health systems are not willing to incur the costs of pharmacogenetic implementation for unproven clinical benefit. However, available germline genetic information should be considered when making treatment decisions.[14] In anticipation of a time when genetic information is available for many patients, perhaps as a result of the proliferation of direct-to-consumer genotyping,[15] the pharmacogenetic community has developed evidence-based pharmacogenetic treatment guidelines.[3,16]

In oncology, there has been a tremendous expansion in the availability of genetic information as a result of the proliferation of tumor sequencing programs that use somatic genetic information to personalize selection of targeted cancer treatments.[17] Although some programs analyze only the somatic genome, others have found that matched germline analysis improves quality control[18] and enables simultaneous assessment of familial predisposition to cancer.[19] This creates a unique situation in which germline genetic information for clinically relevant pharmaco-genes is freely available.[20] However, the opportunity to use these data in clinical practice has not yet been capitalized upon.

The Michigan Oncology Sequencing (MI-Oncoseq) program at The University of Michigan Comprehensive Cancer Center (UMCCC) performs targeted sequencing of somatic and matched germline DNA, in addition to somatic whole-transcriptome analysis.[21] The targeted sequencing panel includes approximately 100 pharmacogenes that could be used to provide evidence-based pharmacogenetic treatment recommendations, if the accuracy of the germline genetic determinations are verified. Our primary objective was to confirm the reliability of germline genotype determinations made by in-house MI-Oncoseq sequencing for polymorphisms in cancer-relevant pharmacogenes. After retrospectively assessing the proportion of MI-Oncoseq patients who carried actionable phenotypes and received a drug relevant to their genotype status, we then reviewed treatment outcomes in these patients to assess the clinical usefulness of integrating germline pharmacogenetics into MI-Oncoseq.

METHODS

MI-Oncoseq Patients

The primary objective of Mi-Oncoseq is to provide precision cancer treatment recommendations based on profiling of the tumor and the patient. Detailed study information including inclusion and exclusion criteria, data analysis and processing, and return of results has been previously published.[21–23] Briefly, patients treated at UMCCC with refractory tumors are invited to participate in a research protocol in which they provide tumor and blood samples for matched genetic sequencing, among other somatic analyses.

Patients sign informed consent, including an opt-out option for germline information. Since the initiation of MI-Oncoseq, the DNA sequencing platform has transitioned from whole-exome sequencing to a targeted exon sequencing panel, and sequencing methods have been previously described in detail.[21,23] This targeted panel sequences primarily exonic regions of approximately 1,700 genes, including approximately 100 pharmacogenes selected based on curation within PharmGKB (Appendix Table A1). The Onco1700 has gone through several updates as sequencing issues have been identified and corrected. Patients for whom germ-line genetic information was available from the fourth version of Onco1700 (Onco1700_V4; Onco1500 v4_160111_HG19_OncoPanelV4_ XC_EZ_HX1) as of December 28, 2016, were included in the retrospective analysis of MI-Oncoseq germline pharmacogenetics.

Onco1700 Genotype Determinations

The genetic data for 21 single-nucleotide polymorphisms (SNPs) in five cancer-related pharmacogenes were collected: *TPMT*: *2 (rs1800462), *3B (rs1800460), *3C (rs1142345), and *4 (rs1800584); *DPYD*: *2 (rs3918290), *13 (rs55886062), and rs67376798 (no * designation); *CYP2C19*: *2 (rs4244285), *3 (rs4986893), *4 (rs28399504), *5 (rs56337013), *6 (rs72552267), *7 (rs72558186), *8 (rs41291556), and *17 (rs12248560); *CYP3A5*: *3 (rs776746), *6 (rs10264272), and *7 (rs41303343); and *UGT1A1*: *6 (rs4148323), *28 (rs8175347), and *80 (rs887829). These five genes were selected based on their relevance to cancer treatment or supportive care, likelihood of clinician interest in prospective implementation of the gene-drug pairs, and existence of evidence-based treatment guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC)[16] or the Dutch Pharmacogenetics Working Group (DPWG).[3] The polymorphisms in each gene were selected based on the validated variant lists within each guideline. Note that *UGT1A1*28* (rs8175347) and *80 (rs887829) were both included to represent the *UGT1A1*28* geno-type, because they are highly linked, and *80 is often substituted for *28 as a result of its relative ease of genotyping.[24] The MI-Oncoseq germ-line genetic database was screened to identify all variant calls at any of these polymorphisms for all patients sequenced on Onco1700_V4, and variant calls were compiled in a single data set for further analysis.

Confirmatory Genotyping

Genotype determinations from Onco1700 were manually screened for potentially unreliable calls by assessing standard sequencing quality control parameters, including read depth. Reliable genotype determinations were then screened to identify patients to send for Clinical Laboratory Improvement Amendments–approved confirmatory genotyping. Up to five samples that were heterozygous and five that were homozygous variant for each polymorphism were selected, while attempting to minimize the total number of samples regenotyped. An iterative manual process was undertaken in which every sample was selected for all polymorphisms with   five occurrences. In the second stage, polymorphisms with > five but < 30 occurrences were selected, by prioritizing patients who also carried a variant at one of the common SNP positions. Finally, as many samples as necessary were selected that carried these common variants so that each variant was represented in five samples.

Selected samples were sent for College of American Pathologists–accredited, Clinical Laboratory Improvement Amendments–approved genotyping at Genelex Laboratories (Seattle, WA). Genotypes were obtained using a laboratory-developed, multiplex polymerase chain reaction–based test followed by single base primer extension for variant detection by mass spectrometry (MassArray Analyzer 4 System; Agena Bioscience, San Diego, CA). Analytic sensitivity and specificity was > 99%.[25] All samples were genotyped for all polymorphisms of interest, and this was conducted blinded to the Onco1700 genotype determinations. The Onco1700 genotype determinations were compared with the Genelex confirmatory genotype determinations to assess concordance. Percent concordance for each SNP was calculated as the number of concordant genotypes divided by the total number of samples compared.

Retrospective Analysis of Clinically Actionable Phenotypes

Patient genotypes were translated to activity phenotypes based on the appropriate CPIC[26] or DPWG[3] guidelines (Appendix Table A2). Clinically actionable phenotypes were defined on the basis of guideline recommendations to adjust dose or select an alternative drug. Genotype data for *UGT1A1*28* (*80) and *CYP3A5*3* was only available for the 25 samples sent for confirma-tory genotyping; therefore, these patients and genotypes were included in assessments of clinical usefulness. The electronic medical records for all patients with actionable phenotypes were screened to determine whether they received the relevant drugs. Medical record screening was performed using an automated screening tool (EMERSE[27]) that searches the text of notes in MiChart, the version of EPIC used at Michigan Medicine. Text used to screen the medical records included all generic and brand drug names and commonly used acronyms (eg, FU for fluorouracil).

A pharmacy student, in consultation with a pharmacist with oncology pharmacogenomic expertise, manually reviewed the electronic medical record for each patient who had an actionable pheno-type and received the relevant drug to determine whether a treatment modification would have been recommended had this genetic information been available to the clinician before treatment initiation. Treatment outcomes relevant to the identified gene-drug interactions were also manually evaluated. Descriptive statistical results for this analysis include the percentages of patients with actionable phenotypes, those who received the relevant drug, and those who would have been eligible for a pharmacogenetic treatment recommendation based on evidence-based guidelines.

RESULTS

MI-Oncoseq Patients and Confirmatory Genotyping

A search of all patients with germline sequence data available from Onco1700_V4 identified 115 patients. These patients represent the diversity of the MI-Oncoseq cohort, including adult (n = 82; 71%) and pediatric patients (n = 33; 29%) who were evenly divided between male (n = 58; 50%) and female sex (n = 57; 50%), were primarily white (n = 97; 84%), and had a variety of solid (n = 80; 70%) and liquid tumor types (n = 35; 30%).

No genotype determinations for *CYP3A5*3*, *UGT1A1*28*, or *UGT1A1*80* were made by Onco1700 because of low read depth (*CYP3A5*3* and *UGT1A1*80*) or sequence repeat misalignment (*UGT1A1*28*); therefore, these polymorphisms were excluded from genotype concordance analyses. Across the 18 remaining SNPs, a total of 139 variant calls were made in these 115 samples, and nine unique SNPs were detected in at least one patient (Table 1). No variants were detected for the remaining nine SNPs.

Using the previously described selection process, residual germline DNA samples from 25 patients were sent for confirmatory genotyping. All variant determinations made by Onco1700 were confirmed, and no variant genotypes were identified by confirmatory genotyping that were not detected by Onco1700. Therefore, confirmatory genotyping was 100% concordant with Onco1700 genotype determinations.

Retrospective Analysis of Clinically Actionable Phenotypes

A determination of clinically actionable phenotype, based on CPIC criteria, was made for each patient for each gene for which he or she had usable genotype data (ie, n = 25 for *CYP3A5* and *UGT1A1*). The number and percentage of patients carrying actionable phenotypes for each gene are listed in Table 2. The frequency of actionable phenotypes was highest for *CYP2C19* (43.5%), followed by *CYP3A5* (20%), *UGT1A1* (20%), and *TPMT* (13%), and lowest for *DPYD* (3.5%), as expected.

The electronic medical record for each patient carrying an actionable phenotype was screened for relevant drugs. Initial screening identified seven patients with actionable phenotypes whose medical records contained mention of a relevant drug. For four of these seven patients, having genetic information would not have led to a treatment recommendation. One patient with *CYP2C19* rapid metabolizer phenotype never received voriconazole; two patients with *CYP2C19* rapid metabolizer phenotype received voriconazole treatment, but they were pediatric patients, so no dose adjustment is recommended per CPIC guidelines[5]; and one patient with *UGT1A1* poor metabolizer phenotype treated with irinotecan received a standard pediatric dose (49 mg/m^2), which is below the recommended threshold for dose adjustment based on DPWG guidelines.[3]

Three patients with actionable phenotypes who received the relevant drug, in whom a treatment recommendation could have been made, were identified. Two pediatric patients diagnosed with lymphoblastic leukemia had *TPMT* genotype ordered from an outside laboratory and were determined to be intermediate metabolizers. Per Children's Oncology Group protocol, both patients started mercaptopurine at standard oral dose (75 mg/m^2 per day). One patient's dose was held after 5 days because of neutropenia (absolute neutrophil count [ANC], 200), and the second patient's dose was reduced to 70% of the standard dose after 7 days because of neutropenia (ANC, 500). The third patient was diagnosed with pancreatic adenocarcinoma. The patient's *DPYD* genotype was available within MI-Oncoseq, but the intermediate metabolizer phenotype was not known by the clinical team. The patient received standard-dose FOLFIRI (FU, leucovorin, and irinotecan), including FU continuous infusion at 2,400 mg/m^2, which caused moderate neutropenia (ANC, 900) but otherwise no notable toxicity.

DISCUSSION

Despite the discovery and validation of germline genotypes that predict treatment outcomes, pharmacogenetics has been slow to be implemented into clinical practice.[28–30] There are many challenges to pharmacogenetic implementation,[10] but perhaps the primary challenge is the current lack of evidence of clinical utility to justify the upfront cost of establishing a pharmacogenetic service.[31,32] In anticipation of a future in which genomic information is more readily available, CPIC and other groups have published evidence-based treatment recommendations for patients with known genotypes.[3,16] There is a unique opportunity to integrate evidence-based pharmacogenetic treatment into tumor sequencing programs that analyze germline genetic information,[20] such as the MI-Oncoseq program at UMCCC.[21] The objective of this analysis was to confirm the accuracy of germ-line genotype determinations produced during MI-Oncoseq sequencing and then to retrospectively assess the clinical usefulness of integrating pharmacogenetics into MI-Oncoseq.

Attempted confirmatory genotyping of 21 clinically actionable SNPs in five cancer-relevant pharmacogenes confirmed genotyping accuracy for common and uncommon variants in three genes (*TPMT*, *DPYD*, and *CYP2C19*) but revealed an inability to genotype common variants in *CYP3A5* and *UGT1A1*. This finding is easily explained by the targeted exonic coverage of Onco1700 and the location of these polymorphisms at a splice site (*CYYP3A5*3*, rs776746) and in the promoter region (*UGT1A1*28*, rs8175347). Other variants that were not detected are extremely rare, and several have not been found in white patients.

Prior studies have estimated that > 90% of the population has an actionable phenotype of at least one candidate gene[33,34]; however, this is only relevant if the patient is treated with the drug of interest and the guidelines apply to the patient. In our cohort, 4% (five of 115) of patients had a potentially actionable phenotype in *CYP2C19*, *TPMT*, or *DPYD* and received the relevant drug, and in 2.6% (three of 115) of patients, a guideline-based treatment recommendation could have been made.

Manual review of these three patients identified several interesting findings. Despite *TPMT* genotype information available at the time of treatment, two patients with heterozygous genotypes initiated treatment at standard mercaptopurine doses, per the Children's Oncology Group protocols on which they were enrolled. CPIC recommends a preemptive 30% to 70% dose decrease with enhanced monitoring and titration based on tolerability.[1] Although germline *TPMT* determination from MI-Oncoseq would not have changed these patients' treatment in any way, it would have prevented external genetic testing, resulting in cost savings to the health system. The third patient carried a *DPYD* geno-type that confers risk of severe toxicity with FU, which was not known at the time of treatment. CPIC guidelines recommend a preemptive dose reduction of 50% with monitoring and titration.[2] Although it is impossible to attribute toxicity to any single factor, it is interesting that two patients experienced toxicity requiring a reactive dose reduction, which may have been prevented if care had been based on CPIC guidelines.

The 2% to 4% absolute increase in the proportion of patients with clinically actionable findings from germline pharmacogenetics represents a minimal estimate, because the EMERSE screening tool does not automatically screen prescribing data, and there is some chance that a patient with an actionable phenotype received a relevant medication that was never mentioned in a clinical note. Regardless, this represents a meaningful increase in the clinically actionable findings from our tumor sequencing program.[35] In addition to its usefulness for quality control,[18,36] matched germline analysis identifies validated cancer predisposition variants in an estimated 15% of patients.[19] Several tumor sequencing programs, including MI-Oncoseq,[21] use their matched germline DNA for this purpose.[37,38] Our results represent a critical first step toward integration of germline pharmacogenetics into MI-Oncoseq.

Although several programs have reported that pharmacogenetics is considered in their decision making,[39,40] we are not aware of any detailed reports of the integration of pharmacogenetics into these programs. A summary of the experience of the Precision in Pediatric Sequencing Program at Columbia University Medical Center mentioned that pharmacogenetic investigation detected clinically meaningful variants in *UGT1A1*.[41] Although the *UGT1A1*28* variant was reported in two patients, according to guideline recommendations, only the patient who was homozygous for *UGT1A1*28* had a clinically actionable phenotype.[3] The implication of drug sensitivity was noted; however, no information was provided on how this information would be used to inform irinotecan treatment decisions[3] or how the incidental finding of Gilbert's syndrome would be conveyed to the patient.[42,43]

Generalization of our estimate of the proportion of patients who could benefit from pharmacogenetic implementation in other tumor sequencing programs is challenging for several reasons. First, there are differences in the frequencies of clinically actionable alleles or phenotypes among racial cohorts.[44] Additionally, institutional differences in the distribution of tumors that are treated and sent for sequencing could dramatically affect this estimate. Furthermore, the potential utility of this approach is highly dependent on when the sequencing occurs. Many programs sequence primarily refractory tumors, at which time most standard treatment has already been exhausted. Pharmacogenetic integration would be most beneficial in programs that sequence tumors early in treatment, particularly at institutions that treat many pediatric patients with ALL.

The proportion of patients who would benefit from pharmacogenetic implementation could be increased in several ways. The next update of Onco1700 will include targeted sequencing coverage of *CYP3A5*3*, *UGT1A1*28*, and *UGT1A1*80*. Another gene that will be included in the next update is *NUDT15*, which contributes to mercaptopurine toxicity[45,46] and is being added to the CPIC thiopurine guidelines. Another high-priority target is *CYP2D6*, which has CPIC guidelines for several drugs used commonly in cancer supportive care, including narcotic anal-gesics,[47] 5-hydroxytryptamine-3 antiemetics,[6] and antidepressants.[48] Unfortunately, *CYP2D6* had to be excluded from this initial analysis because of the complexity of assigning genotype determinations from sequencing data[49] and making genotype-based clinical recommendations.[50]

In addition to genotyping cost, prospective implementation of pharmacogenetics requires substantial upfront investment to build and maintain clinical decision support within the electronic health record, hire or train individuals with pharmacogenetic expertise, and provide clinician and patient education. Pharmacogenetic implementation within tumor sequencing programs that analyze matched germline DNA is particularly efficient because there is no geno-typing cost and the bioinformatic workflow to detect actionable germline phenotypes can be integrated into the existing infrastructure. However, substantial investment is still required to integrate pharmacy and pharmacogenomic expertise[51] into the multidisciplinary precision medicine tumor board that oversees genetically informed treatment decision making. In the MI-Oncoseq model, pharmacists will work alongside medical oncologists and genetic counselors,[52] providing pharmacogenetic expertise and education. Additional work is necessary to develop infrastructure to embed active clinical decision support into the electronic health record so that actionable phenotypes can be stored and used indefinitely.[7,11]

In conclusion, Onco1700 produces reliable germline pharmacogenetic information for three clinically relevant pharmacogenes (*TPMT*, *DPYD*, and *CYP2C19*). Prospective implementation of pharmacogenetics within MI-Oncoseq will enable evidence-based treatment recommendations in 2% to 4% of MI-Oncoseq patients. Integration of germline pharmacogenetics into a tumor sequencing program is a uniquely efficient opportunity to maximize the clinical benefit of genomic testing, taking another step toward precision medicine for patients with cancer.

ACKNOWLEDGMENT

Appendix

Table A1.

Pharmacogenes Sequenced on Onco1700

Pharmacogene
ABCA1
ABCB1
ABCB11
ABCC2
ABCG1
ABCG2

Pharmacogene
ACE
ADRB1
ADRB2
AHR
ALOX5
APOA1
AR1D5B
BCL2L11
BDNF
CACNA1C
CACNA1S
CACNB2
CES1
CES2
COMT
CRHR1
CYP1A2
CYP2A6
CYP2B6
CYP2C19
CYP2C8
CYP2C9
CYP2D6
CYP2J2
CYP2R1
CYP3A4
CYP3A5
CYP4F2
DBH
DPYD
DRD1
DRD2
EGFR
ESR1
F5
FKBP5
G6PD
GGCX
GLCCI1

Pharmacogene
GRK4
GRK5
GSTP1
GSTT1
HLA-B
HLA-DQB3
HMGCR
HSD11B2
HTR1A
HTR2A
KCNH2
KCNJ11
LDLR
MAOA
MTHFR
NAT2
NPPB
NPR1
NQO1
NR1I2
NR3C1
NR3C2
NTRK2
P2RY1
P2RY12
PEAR1
POR
PTGIS
PTGS1
PTGS2
RYR1
RYR2
SCN5A
SLC15A2
SLC19A1
SLC22A1
SLC22A2
SLC22A3
SLC22A6

Pharmacogene
SLC47A1
SLC47A2
SLC6A3
SLC6A4
SLCO1A2
SLCO1B1
SLCO1B3
SLCO2B1
SULT1A1
TBXAS1
TCL1A
TPMT
TYMS
UGT1A1
UGT1A4
VDR
VKORC1
ZNF423

Table A2.

Results of Confirmatory Genotyping

Genotype	Phenotype	No. in Onco1700[†]	No. in Genelex	Concordant (%)
TPMT				
*1/*1	NM	100	17	100
*1/*2	IM	2	2	100
*1/*3A[‡]	IM	10	5	100
*1/*3C[§]	IM	3	1	100
DPYD				
*1/*1	NM	111	21	100
*1/*2	IM	2	2	100
*1/rs67376798	IM	2	2	100
CYP2C19				
*17/*17	UM	10	5	100
*1/*17	RM	36	3	100
*1/*1	NM	20	6	100
*1/*2	IM	32	4	100
*1/*4	IM	1	1	100
*2/*17	IM	12	2	100

Genotype	Phenotype	No. in Onco1700[†]	No. in Genelex	Concordant (%)
*2/*2	PM	4	4	100
CYP3A5				
*1/*3	IM	NA	4	NA
*1/*6	IM	NA	1	100
*3/*3	PM	NA	18	NA
*3/*6	PM	NA	2	NA
UGT1A1				
*1/*1	NM	NA	11	NA
*1/*80	IM	NA	9	NA
*80/*80	PM	NA	5	NA

Abbreviations: IM, intermediate metabolizer; NA, not applicable; NM, normal metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer.

[†]Some genotypes (indicated by NA) were not known because of sequencing failure of *CYPA5*3*, *UGT1A1*28*, and *UGT1A1*80* on the Onco1700.

[‡]*3A is a haplotype containing *3B and *3C. Neither Onco1700 sequencing or Genelex genotyping can differentiate between a *1/*3A heterozygous patient or the (extremely rarely expected) *3B/*3C compound heterozygous patient.

[§]Because *3C is a component of *3A haplotype, not all *1/*3C patients were sent for confirmatory genotyping.

REFERENCES

1. Relling MV, Gardner EE, Sandborn WJ, et al.: Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clin Pharmacol Ther 93:324–325, 2013 [PubMed: 23422873]

2. Caudle KE, Thorn CF, Klein TE, et al.: Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther 94:640–645, 2013 [PubMed: 23988873]

3. Swen JJ, Nijenhuis M, de Boer A, et al.: Pharmacogenetics: From bench to byte—An update of guidelines. Clin Pharmacol Ther 89:662–673, 2011 [PubMed: 21412232]

4. Birdwell KA, Decker B, Barbarino JM, et al.: Clinical pharmacogenetics implementation consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. Clin Pharmacol Ther 98:19–24, 2015 [PubMed: 25801146]

5. Moriyama B, Obeng AO, Barbarino J, et al.: Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP2C19 and voriconazole therapy. Clin Pharmacol Ther [epub ahead of print on December 16, 2016]

6. Bell GC, Caudle KE, Whirl-Carrillo M, et al.: Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 genotype and use of ondansetron and tropisetron. Clin Pharmacol Ther 102:213–218, 2017 [PubMed: 28002639]

7. Hoffman JM, Haidar CE, Wilkinson MR, et al.: PG4KDS: A model for the clinical implementation of pre-emptive pharmacogenetics. Am J Med Genet C Semin Med Genet 166C:45–55, 2014 [PubMed: 24619595]

8. Patel JN: Cancer pharmacogenomics, challenges in implementation, and patient-focused perspectives. Pharm Genomics Pers Med 9:65–77, 2016

9. Volpi S, Bult CJ, Chisholm RL, et al.: Research directions in the clinical implementation of pharmacogenomics: An overview of US programs and projects. Clin Pharmacol Ther 103:778–786, 2018 [PubMed: 29460415]

10. Shuldiner AR, Relling MV, Peterson JF, et al.: The Pharmacogenomics Research Network Translational Pharmacogenetics Program: Overcoming challenges of real-world implementation. Clin Pharmacol Ther 94:207–210, 2013 [PubMed: 23588301]

11. Dunnenberger HM, Crews KR, Hoffman JM, et al.: Preemptive clinical pharmacogenetics implementation: Current programs in five US medical centers. Annu Rev Pharmacol Toxicol 55:89–106, 2015 [PubMed: 25292429]

12. Caudle KE, Gammal RS, Whirl-Carrillo M, et al.: Evidence and resources to implement pharmacogenetic knowledge for precision medicine. Am J Health Syst Pharm 73:1977–1985, 2016 [PubMed: 27864205]

13. Gillis NK, Innocenti F: Evidence required to demonstrate clinical utility of pharmacogenetic testing: The debate continues. Clin Pharmacol Ther 96:655–657, 2014 [PubMed: 25399714]

14. Altman RB: Pharmacogenomics: "noninferiority" is sufficient for initial implementation. Clin Pharmacol Ther 89:348–350, 2011 [PubMed: 21326263]

15. Filipski KK, Murphy JD, Helzlsouer KJ: Updating the landscape of direct-to-consumer pharmacogenomic testing. Pharm Genomics Pers Med 10:229–232, 2017

16. Relling MV, Klein TE: CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. Clin Pharmacol Ther 89:464–467, 2011 [PubMed: 21270786]

17. Heuckmann JM, Thomas RK: A new generation of cancer genome diagnostics for routine clinical use: Overcoming the roadblocks to personalized cancer medicine. Ann Oncol 26:1830–1837, 2015 [PubMed: 25899787]

18. Jones S, Anagnostou V, Lytle K, et al.: Personalized genomic analyses for cancer mutation discovery and interpretation. Sci Transl Med 7:283ra53, 2015

19. Schrader KA, Cheng DT, Joseph V, et al.: Germline variants in targeted tumor sequencing using matched normal DNA. JAMA Oncol 2:104–111, 2016 [PubMed: 26556299]

20. Hertz DL, McLeod HL: Integrated patient and tumor genetic testing for individualized cancer therapy. Clin Pharmacol Ther 99:143–146, 2016 [PubMed: 26537014]

21. Roychowdhury S, Iyer MK, Robinson DR, et al.: Personalized oncology through integrative high-throughput sequencing: A pilot study. Sci Transl Med 3:111ra121, 2011

22. Mody RJ, Wu YM, Lonigro RJ, et al.: Integrative clinical sequencing in the management of refractory or relapsed cancer in youth. JAMA 314:913–925, 2015 [PubMed: 26325560]

23. Robinson DR, Wu YM, Lonigro RJ, et al.: Integrative clinical genomics of metastatic cancer. Nature 548:297–303, 2017 [PubMed: 28783718]

24. Gammal RS, Court MH, Haidar CE, et al.: Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for UGT1A1 and atazanavir prescribing. Clin Pharmacol Ther 99:363–369, 2016 [PubMed: 26417955]

25. Elliott LS, Henderson JC, Neradilek MB, et al.: Clinical impact of pharmacogenetic profiling with a clinical decision support tool in polypharmacy home health patients: A prospective pilot randomized controlled trial. PLoS One 12:e0170905, 2017 [PubMed: 28151991]

26. Caudle KE, Dunnenberger HM, Freimuth RR, et al.: Standardizing terms for clinical pharmacogenetic test results: Consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC). Genet Med 19:215–223, 2017 [PubMed: 27441996]

27. Hanauer DA, Mei Q, Law J, et al.: Supporting information retrieval from electronic health records: A report of University of Michigan's nine-year experience in developing and using the Electronic Medical Record Search Engine (EMERSE). J Biomed Inform 55:290–300, 2015 [PubMed: 25979153]

28. Relling MV, Evans WE: Pharmacogenomics in the clinic. Nature 526:343–350, 2015 [PubMed: 26469045]

29. Weitzel KW, Alexander M, Bernhardt BA, et al.: The IGNITE network: A model for genomic medicine implementation and research. BMC Med Genomics 9:1, 2016 [PubMed: 26729011]

30. Luzum JA, Pakyz RE, Elsey AR, et al.: The Pharmacogenomics Research Network Translational Pharmacogenetics Program: Outcomes and metrics of pharmacogenetic implementations across diverse healthcare systems. Clin Pharmacol Ther 102:502–510, 2017 [PubMed: 28090649]

31. Pirmohamed M: Acceptance of biomarker-based tests for application in clinical practice: Criteria and obstacles. Clin Pharmacol Ther 88:862–866, 2010 [PubMed: 20981006]

32. Khoury MJ: Dealing with the evidence dilemma in genomics and personalized medicine. Clin Pharmacol Ther 87:635–638, 2010 [PubMed: 20485318]

33. Van Driest SL, Shi Y, Bowton EA, et al.: Clinically actionable genotypes among 10,000 patients with preemptive pharmacogenomic testing. Clin Pharmacol Ther 95:423–431, 2014 [PubMed: 24253661]

34. Ji Y, Skierka JM, Blommel JH, et al.: Preemptive pharmacogenomic testing for precision medicine: A comprehensive analysis of five actionable pharmacogenomic genes using next-generation DNA sequencing and a customized CYP2D6 genotyping cascade. J Mol Diagn 18:438–445, 2016 [PubMed: 26947514]

35. Gornick MC, Cobain E, Le LQ, et al.: Oncologists' use of genomic sequencing data to inform clinical management. JCO Precis Oncol doi:10.1200/PO.17.00122

36. Cabanillas R, Diñeiro M, Castillo D, et al.: A novel molecular diagnostics platform for somatic and germline precision oncology. Mol Genet Genomic Med 5:336–359, 2017 [PubMed: 28717660]

37. Zehir A, Benayed R, Shah RH, et al.: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 23:703–713, 2017 [PubMed: 28481359]

38. Stockley TL, Oza AM, Berman HK, et al.: Molecular profiling of advanced solid tumors and patient outcomes with genotype-matched clinical trials: The Princess Margaret IMPACT/COMPACT trial. Genome Med 8:109, 2016 [PubMed: 27782854]

39. Uzilov AV, Ding W, Fink MY, et al.: Development and clinical application of an integrative genomic approach to personalized cancer therapy. Genome Med 8:62, 2016 [PubMed: 27245685]

40. Radovich M, Kiel PJ, Nance SM, et al.: Clinical benefit of a precision medicine based approach for guiding treatment of refractory cancers. Oncotarget 7:56491–56500, 2016 [PubMed: 27447854]

41. Oberg JA, Glade Bender JL, Sulis ML, et al.: Implementation of next generation sequencing into pediatric hematology-oncology practice: Moving beyond actionable alterations. Genome Med 8:133, 2016 [PubMed: 28007021]

42. Bosma PJ, Chowdhury JR, Bakker C, et al.: The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 333:1171–1175, 1995 [PubMed: 7565971]

43. Pasternak AL, Ward KM, Luzum JA, et al.: Germline genetic variants with implications for disease risk and therapeutic outcomes. Physiol Genomics 49:567–581, 2017 [PubMed: 28887371]

44. Patel JN: Cancer pharmacogenomics: Implications on ethnic diversity and drug response. Pharmacogenet Genomics 25:223–230, 2015 [PubMed: 25751395]

45. Moriyama T, Yang YL, Nishii R, et al.: Novel variants in NUDT15 and thiopurine intolerance in children with acute lymphoblastic leukemia from diverse ancestry. Blood 130:1209–1212, 2017 [PubMed: 28659275]

46. Moriyama T, Nishii R, Perez-Andreu V, et al.: NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet 48:367–373, 2016 [PubMed: 26878724]

47. 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 91:321–326, 2012 [PubMed: 22205192]

48. Hicks JK, Bishop JR, Sangkuhl K, et al.: Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 and CYP2C19 genotypes and dosing of selective serotonin reuptake inhibitors. Clin Pharmacol Ther 98:127–134, 2015 [PubMed: 25974703]

49. Numanagi I, Maliki S, Pratt VM, et al.: Cypiripi: Exact genotyping of CYP2D6 using high-throughput sequencing data. Bioinformatics 31:i27–i34, 2015 [PubMed: 26072492]

50. Hicks JK, Swen JJ, Gaedigk A: Challenges in CYP2D6 phenotype assignment from genotype data: A critical assessment and call for standardization. Curr Drug Metab 15:218–232, 2014 [PubMed: 24524666]

51. Walko C, Kiel PJ, Kolesar J: Precision medicine in oncology: New practice models and roles for oncology pharmacists. Am J Health Syst Pharm 73:1935–1942, 2016 [PubMed: 27864201]

52. Mills R, Haga SB: Clinical delivery of pharmacogenetic testing services: A proposed partnership between genetic counselors and pharmacists. Pharmacogenomics 14:957–968, 2013 [PubMed: 23746189]

Table 1.

Genotype Determinations by Onco1700 for Clinically Actionable SNPs (n = 21)

rsID	Allele	No. Heterozygous	No. Homozygous	Sequencing Notes
TPMT				
rs1800462	*2	2	0	
rs1800460	*3B	10	0	
rs1142345	*3C	13	0	
rs1800584	*4	0	0	
DPYD				
rs3918290	*2	2	0	
rs55886062	*13	0	0	
rs67376798	NA	2	0	
CYP2C19				
rs4244285	*2	44	4	
rs4986893	*3	0	0	
rs28399504	*4	1	0	
rs56337013	*5	0	0	
rs72552267	*6	0	0	
rs72558186	*7	0	0	
rs41291556	*8	0	0	
rs12248560	*17	48	10	
CYP3A5				
rs776746	*3	0	0	Low read depth
rs10264272	*6	3	0	
rs41303343	*7	0	0	
UGT1A1				
rs4148323	*6	0	0	
rs8175347	*28	0	0	Repeat misalignment
rs887829	*80	0	0	Low read depth

Abbreviations: NA, not applicable; SNP, single-nucleotide polymorphism.

Table 2.

Clinically Actionable Genotypes in MI-Oncoseq Patients

Gene	Relevant Drug	Actionable Phenotype	No. With Actionable Phenotype (%)	No. With Actionable Phenotype and Drug of Interest* (%)	No. With Actionable Phenotype and Possible Treatment Recommendation (%)
TPMT	Mercaptopurine, thioguanine, azathioprine	IM	15 (13)[†]	2 (1.7)[†]	2 (1.7)
		PM	0	0	0
DPYD	Fluourouracil, capecitabine	IM	4 (3.5)	1 (0.9)	1 (0.9)
		PM	0	0	0
CYP2C19	Voriconazole	UM	10 (8.7)	0	0
		RM	36 (31.3)	3 (2.6)	0
		PM	4 (3.5)	0	0
CYP3A5[‡]	Tacrolimus	NM	0 (0)[§]	0 (0)[§]	0
		IM	5 (20)	0	0
UGT1A1[‡]	Irinotecan	PM	5 (20)	1 (4.0)	0

Abbreviations: IM, intermediate metabolizer; MI-Oncoseq, Michigan Oncology Sequencing; NM, normal metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.

*
Drug of interest was mentioned in medical record but not necessarily received by patient.

[†]
Of 115 patients.

[‡]
Phenotype determinations made based on results of confirmatory genotyping for 25 patients.

[§]
Of 25 patients.