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Review

Pharmacogenomics and ALL treatment: How to optimize therapy

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ABSTRACT

Inherited genetic variations may alter drug sensitivity in patients with acute lymphoblastic leukemia, predisposing to adverse treatment side effects. In this review, we discuss evidence from children and young adults with acute lymphoblastic leukemia to review the available pharmacogenomic data with an emphasis on clinically actionable and emerging discoveries, for example, genetic variants in thiopurine methyltransferase and *NUDT15* that alter 6-mercaptopurine dosing. We also highlight the need for ongoing pharmacogenomic research to validate the significance of recent findings. Further research in young adults, as well as with novel therapeutics, is needed to provide optimal therapy in future trials.

Introduction

Improvements in outcomes for acute lymphoblastic leukemia (ALL) have been driven in part by the ability to better personalize therapy to reduce toxicity and improve efficacy. Key aspects of this personalization include risk-adapted therapy based on both leukemia genetics, disease response, as well as adapting chemotherapeutic regimens to attempt to minimize toxicity to patients. Chemotherapy can be preemptively modified using pharmacogenomics on the basis of germline genetic variations that influence the responsiveness and toxicity of chemotherapy [1].

Because pharmacogenomics involves the interaction between chemotherapy and inherited genetic variation, it is necessary to understand both the patient-specific genetics influencing treatment response as well as the chemotherapeutic regimen being utilized [2]. In this review, we will highlight data demonstrating genetic variations which may affect the efficacy and toxicity of current treatment regimens for young adults with ALL. While we will highlight recent findings in adult pharmacogenomic studies, we will also note relevant data from pediatric studies using similar chemotherapeutic regimens. Increasing similarity between adult and pediatric trials makes these comparisons meaningful given overlapping chemotherapeutic agents [3-5]. We will divide our review according to the implicated chemotherapy agent and focus on the five drug groups with the most extensive data: 6-mercaptopurine, methotrexate, asparaginase, vincristine, and glucocorticoids.

6-Mercaptopurine

6-Mercaptopurine (6-MP) is a mainstay of maintenance therapy for patients with ALL. It is taken orally daily with doses adjusted based on myelosuppression, the primary toxicity of 6-MP. Mercaptopurine exerts its antileukemic effects through conversion to thioguanine nucleotides (TGNs) which are ultimately incorporated into DNA (DNA-TG) [6]. There, they result in DNA damage and ultimately apoptosis (Fig. 1). In addition to its use in ALL, mercaptopurine is used for patients with inflammatory bowel disease, as is its prodrug azathioprine. A related compound, 6-thioguanine, is also utilized in ALL, typically during intensification phases. While the primary toxicity of both mercaptopurine and thioguanine is myelosuppression, thioguanine is also associated with a risk of venoocclusive disease/sinusoidal obstructive syndrome [7]. In contrast, medication-induced transaminitis is more common with mercaptopurine and is reversible without long-term hepatic injury, likely involving methyl metabolites of thiopurines.

Mercaptopurine is the best characterized pharmacokinetic drug-gene pairing in ALL. Inter-individual differences in tolerance to mercaptopurine resulted in typical dose ranges between 10 mg/m^2 3 days a week to more than 75 mg/m^2 daily, with most patients tolerating 50 to 75mg/m^2 daily [8]. The first genetic variant identified which was associated with decreased tolerance to mercaptopurine was thiopurine methyltransferase (TPMT) [9], a gene whose activity is regulated primarily by coding variants within the gene itself [10]. This association has been identified across diseases

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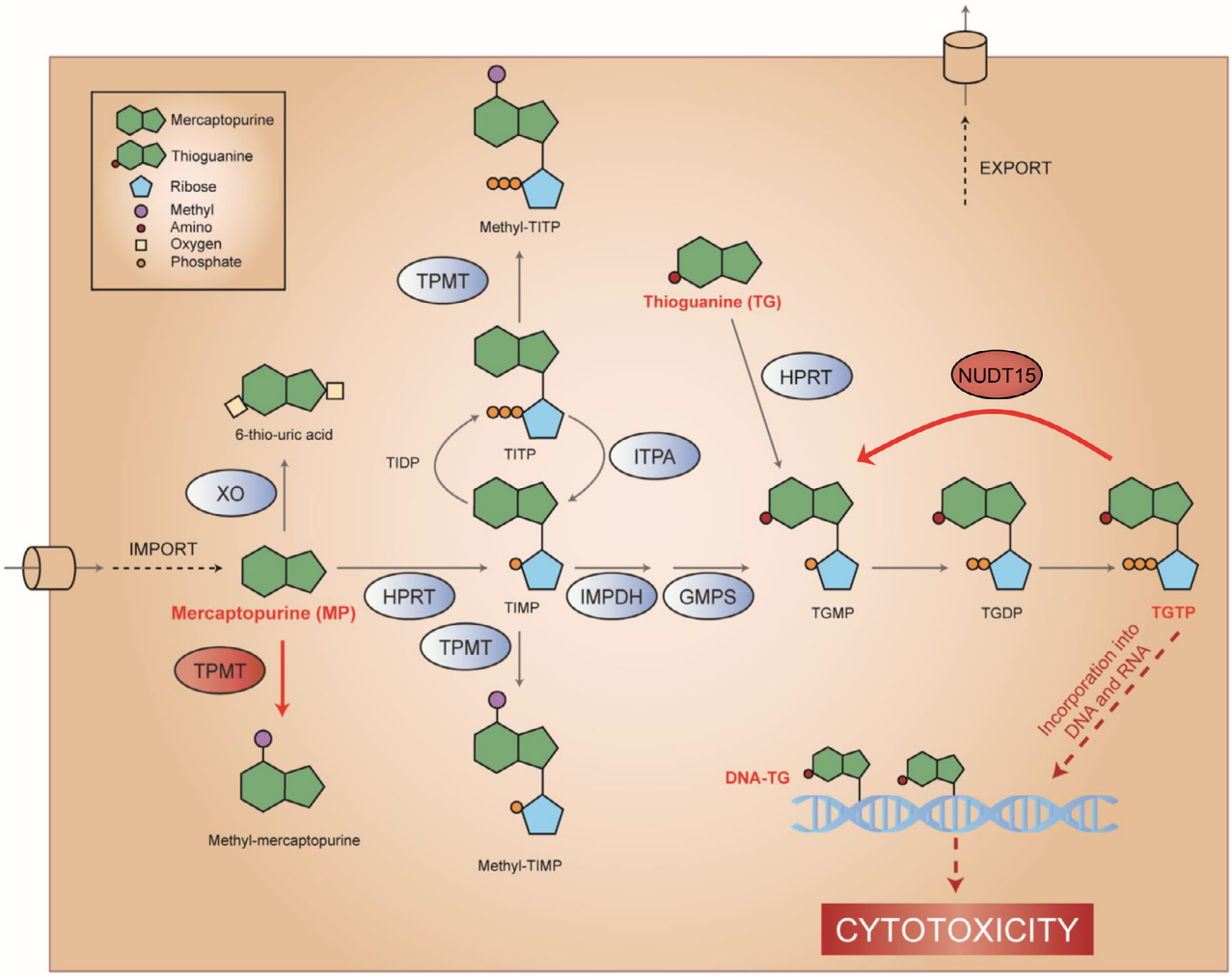

Fig. 1. TPMT and NUDT15 inactivate thiopurines. As prodrugs, thiopurines (mercaptopurine [6MP] and thioguanine [TG]) are enzymatically metabolized to TGTP that is incorporated into DNA resulting in DNA damage and cytotoxicity. TPMT reduces MP cytotoxicity by converting it to inactive methyl-MP, whereas NUDT15 dephosphorylates TGTP and converts it to inactive TGMP. TPMT and NUDT15 are highlighted in red ovals, while MP/TG and their active metabolites (TGTP and DNA.TG) are shown in red text. TPMT = thiopurine methyltransferase.

treated with this drug, including inflammatory bowel disease as well as ALL [11].

TPMT methylates mercaptopurine and its intermediate metabolites; this results in lower thioguanine nucleotide levels [12]. While thioguanine nucleotides have been most directly associated with cellular cytotoxicity, methylated mercaptopurines have been associated with transaminitis during mercaptopurine therapy [13]. Measurement of red blood cell TGN levels is now used as a surrogate for these levels in blasts, as red blood cell (RBC) and blast TPMT activity are highly correlated and red blood cell TGN levels are highly correlated with hematological toxicity [9,14,15]. While no clinical threshold has been established for either efficacy or toxicity in patients with ALL, evaluation of red blood cell thioguanine and methyl mercaptopurine nucleotide levels have been used to monitor adherence to therapy in patients receiving 6-MP [16-18]. Variants in TPMT are present in approximately 4.5% of patients with European ancestry, 7.7% of patients with African Americans, ~2% of Asians, and 5% of Hispanic patients [19-21]. The most common variant allele in European, Hispanic, and Asian patients is the 3A (C>T at rs1800460 and T>C at rs1142345), while the 3C allele (T>C at rs1142345) is the most common variant identified in African Americans (Table 1) [21]. Patients with one known inactivating variant in TPMT (ie, *3A, *3C, and *2) are considered intermediate metabolizers, while patients with 2 inactivating alleles are considered poor metabolizers. All other patients are considered normal metabolizers. Preemptive identification of the genetic variants in children with ALL resulted in reduced periods of myelosuppression as well as a reduction in the risk of secondary acute myeloid leukemia [9,22,23]. Limited adult data also suggests that patients who experience severe hematological toxicity while receiving mercaptopurine frequently carries variations in TPMT [24].

The recent discovery of the association between *NUDT15* variants and mercaptopurine intolerance has expanded the understanding of causal variants driving thiopurine induced myelosuppression. Historical demographic data have suggested that patients of Asian ancestry are more sensitive to 6-MP than patients of European ancestry, with mean tolerated dose approximately 65% of what is observed in other populations [25]. Genome-wide association studies (GWAS) identified variants in *NUDT15* significantly associated with this dose intolerance [25]. These variants are present across genetic ancestries but are relatively less common in patients of European ancestry (0.7%) compared to those of Hispanic or Asian ancestry, in which they are observed in 4.5% to 6.4% and 10% to 12.1% of patients, respectively (Table 1) [21,25]. Similar to patients with only inactivating *TPMT* variations, patients

Table 1
Frequently encountered low-function alleles in *TPMT* and *NUDT15*.
Data for the frequency of *NUDT15* variant alleles in African Americans is unavailable at this time. Adapted from current Clinical Pharmacogenetics Implementation Consortium guidelines [21].

Gene/haplotype	rsID	Allele frequency			
		European	African American	Hispanic	East Asian
TPMT					
*3A	rs1800460, rs1142345	3.4%	0.8%	4.2%	<0.1%
*3C	rs1142345	0.5%	2.4%	0.6%	1.6%
*2	rs267607275	0.2%	0.5%	0.4%	<0.1%
Other nonreference	Various	0.6%	3.9%	0.6%	0.4%
NUDT15					
*2	rs869320766, rs116855232	<0.1%		3.7%	3.5%
*3	rs116855232	0.2%		0.8%	6.1%
*9	rs746071566	<0.1%		<0.1%	<0.1%

TMPT = thiopurine methyltransferase.

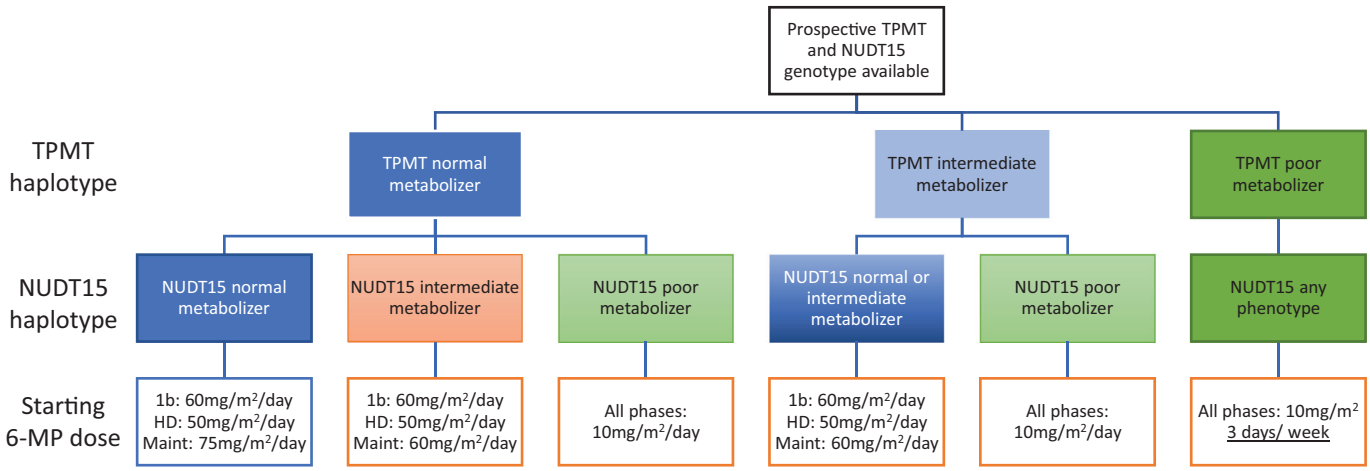

Fig. 2. Genotype guided dose of 6-MP. Starting 6-MP doses in Total 17 (NCT03117751) vary by phase of therapy. Patients with one known inactivating variant in TPMT (ie, *3A, *3C, and *2) or NUDT15 (ie, *2, *3, and *9) are considered intermediate metabolizers for the respective enzyme, while patients with 2 inactivating alleles are considered poor metabolizers. All other patients are considered normal metabolizers. Phases of therapy include late induction (1b), consolidation with high-dose methotrexate (HD), and continuation (Maint). All doses are given daily with the exception of patients with TPMT poor metabolizer genotypes who receive 6-MP only 3 of 7 days each week. TPMT = thiopurine methyltransferase.

with one loss-of-function *NUDT15* variation (ie, *2, *3, and *9) are considered intermediate metabolizers and tolerate 6-MP after a 20% to 30% dose reduction (ie, 50 to 60mg/m^2/day as a starting dose) [21,25]. While rare, *NUDT15* variants in European adults are also associated with myelosuppression [24]. Again similar to TPMT variant patients, those with 2 inactivating mutations are considered poor metabolizers and tolerate less than 10% of the protocol specified dose (ie, 10 mg/m^2/dose 3 to 7 days/ week; Fig. 2).

NUDT15 acts by dephosphorylating thioguanine nucleotides prior to their incorporation into DNA [26]. Once thioguanine nucleotides are incorporated into DNA, they result in DNA damage and ultimately apoptosis. Damaging variants in *NUDT15* result in increased incorporation of thioguanine nucleotides into DNA and thus increased sensitivity to the drug in the form of increased myelosuppression.

Because germline genetic variation in *TPMT* and *NUDT15* are "inherited" by a patients' ALL blasts, dose adjustments to reduce myelotoxicity can be made while maintaining therapeutic efficacy [27,28]. Data from clinical trials in which prospective dose decreases in 6-MP were made in patients with *TPMT* variation indicate similar therapeutic efficacy in patients with *TPMT* variant and wild-type alleles [29]. Preclinical data demonstrate the efficacy of these NUDT15-informed decreases on murine leukemias [30,31]. Within the context of TPMT, host metabolism effects drug exposure such that even when wild-type TPMT leukemias are present in TPMT deficient hosts, therapeutic efficacy is maintained with dose decreased mercaptopurine [27].

In summary, clinical and preclinical data strongly support prospective dose adjustments of 6-MP in the context of *TPMT* or *NUDT15* genetic variations. These recommendations are highlighted in a recently updated guideline from the Clinical Pharmacogenetics Implementation Consortium [21].

Methotrexate

Methotrexate exerts antileukemic efficacy through alterations in the folate metabolism pathway and subsequent alterations in purine generation and DNA synthesis. It is critical to antileukemic therapy and is used in intrathecal injections to control or prevent central nervous system (CNS) leukemia, during interim maintenance periods either in the form of high-dose methotrexate (with leucovorin rescue) or in an escalating IV fashion when combined with asparaginase, the so-called Capizzi regiment. Finally, along with mercaptopurine, it is taken either orally or intravenously weekly as part of maintenance therapy.

The most commonly observed side effects of methotrexate include myelosuppression, mucositis, and hepatic toxicity [4]. In addition, acute renal insufficiency may also be observed, particularly after high-dose administration [32]. More rarely, an acute stroke-like syndrome is observed which is typically transient and resolves within 7 to 10 days [33]. Chronically, leukoencephalopathy and

neurological impairment can be observed both during therapy and into survivorship [34,35].

There have been no genetic variants which have been integrated into preemptive pharmacogenomics testing prior to methotrexate administration. Both GWAS and candidate gene approaches have been used to attempt to identify genetic variants which are associated with methotrexate sensitivity or toxicity. Variants in SLCO1B1 were first identified as being associated with methotrexate clearance and gastrointestinal toxicity in 3 St. Jude treatment cohorts [36]. Variants associated with slower methotrexate clearance were also associated with an increased risk of gastrointestinal toxicity/ mucositis in treatment regimens that did not adjust doses to target a predetermined drug exposure level. In contrast, this association with toxicity was not observed in regimens that specified the desired exposure (rather than a prespecified dose). This suggests that altering drug dosing to account for alterations in clearance mediated by SLCO1B1 variation can ameliorate toxicity. Although genetic variants in SLCO1B1 accounted for greater variation in clearance than sex, ancestry, or age, these variants explained only 11% of the variation in clearance. By comparison, dosing regimen accounted for 17% of the variability in drug clearance.

The association between these variants alleles and methotrexate clearance was validated in an additional study of 1279 patients with ALL [37]. In addition to the lead SLCO1B1 single nucleotide polymorphism (SNP), 2 additional SNPs in this gene were associated with methotrexate clearance after adjusting for the lead SNP. Within this cohort, the SNPs accounted for approximately 2% of the variation in methotrexate clearance in contrast to a 38% difference in clearance associated with a 4-hour versus 24-hour infusion. Notably, the variability associated with genetics was still greater than other treatment factors such as the presence of a delayed intensification phase, age, or sex.

Although the findings of these studies have not led to preemptive pharmacogenetics dose adjustments, they remain significant because they have highlighted the importance of the SLCO1B1 transporter in the clearance of methotrexate. This highlights potential drug-drug interactions that are particularly important around high-dose methotrexate infusions. Notably, proton pump inhibitors, aminopenicillins, and statins may all act as inhibitors of SLCO1B1 and therefore result in increased methotrexate exposure and associated toxicities [38].

In summary, interpatient and intrapatient variability in dosing regimen result in greater differences in toxicity and clearance than genetic variants. Thus, careful monitoring of patients receiving high-dose methotrexate is needed to ensure optimal therapeutic efficacy and minimize toxicity.

Asparaginase

Asparaginase deaminates the amino acids asparagine and, to a lesser extent, glutamine to form aspartic acid and glutamic acid, respectively. This depletes serum asparagine. While most cells can generate asparagine using asparagine synthetase, ALL blasts are unable to do so because they frequently lack asparagine synthetase expression. The use of asparaginase in pediatric ALL regimens is a key difference when compared to conventional adult ALL regimens. The effectiveness of asparaginase in pediatric ALL regimens has been well documented [39-41], and improvements in outcomes for adults with ALL receiving pediatric regimens have been attributed to the addition of asparaginase to these therapies [3]. Common toxicities experienced by patients receiving asparaginase include allergic reaction, thrombosis, hepatic injury, and pancreatitis. Allergic reactions can be either clinically apparent or associated with a subclinical immune response that results in rapid asparaginase clearance ("silent inactivation") and therefore inadequate asparagine depletion.

Asparaginase allergy occurs in between 5 and 15% of patients receiving multiple doses of pegylated asparaginase during ALL therapy [42-44]. Antiasparaginase antibodies are highly correlated with the development of clinical allergy, with almost all patients who experience a clinical allergy having antiasparaginase antibodies [42]. Silent inactivation can also occur in the presence of antibodies and is associated with an inferior therapeutic response [41,45]. Candidate gene studies evaluating major histone compatibility regions have identified the human leukocyte antigen (HLA)DRB1*07:01 haplotype as being associated with the development of asparaginase allergy [46,47]. Patients with this haplotype had a 60% increased risk of developing clinical hypersensitivity and a 2.9-fold increased risk of developing antiasparaginase antibodies [47]. This association was maintained in both patients that received native asparaginase (which was used historically but is no longer commercially available in the United States) as well as pegylated asparaginase which is currently used in frontline trials around the world. The mechanism of this association appears to be increased binding due to alterations in the amino acid sequence in the binding pocket of the HLA-DR. While less frequently seen in the population studied, associations were also observed between HLA-DRB1*04:05 and *04:08 haplotypes.

Evaluations in a European cohort confirmed associations between HLA loci and the development of asparaginase allergy [48]. In that cohort, HLA–DQA1 was associated with an increased risk of asparaginase allergy, although individual patient haplotype's were not assigned. This study also associated a variation in *CNOT3*, a gene associated with major histocompatibility class II expression, with the development of asparaginase allergy. In a multiethnic cohort of patients treated in the United States, variants in *NFATC2* were associated with an increased development of asparaginase allergy [46]. Interestingly, this gene is located on chromosome 21 and is paradoxically inhibited in Down's syndrome due to overexpression of competing repressive genes. This analysis also demonstrated that patients with Down's syndrome have a lower incidence of asparaginase allergy than patients without constitutional trisomy 21.

Asparaginase hepatotoxicity appears to be more common in adults than in children receiving similar ALL therapy [3,4,49]. Attempts to identify genetic associations with asparaginase induced hepatotoxicity have included evaluations of both adult and pediatric cohorts. In one adult cohort, the 55 patients with the rs4880 CC genotype in *SOD2* had a 2.5-fold increase in their risk of grade 3/4 ALT/AST/bilirubin increase compared to the 135 patients with either a CT or TT genotype [50]. Separately, GWAS in pediatric patients identified a variant in *PNPLA3* as being associated with the development of hepatotoxicity during ALL induction [51]. The identified variant (rs738409) has also been associated with the development of fatty liver disease in adult populations. Although not clinically actionable, these data, combined with data demonstrating fat accumulation as a mechanism of asparaginase induced hepatic injury [52,53], suggest potential pathways underlying therapy toxicity which may be amenable to intervention.

Extensive work has been undertaken to identify genetic risk factors for the development of asparaginase associated pancreatitis. Identified variants linked to increased risk of pancreatitis have varied across cohorts. A candidate gene evaluation of French-Canadian children treated on Dana-Farber protocols identified the *1 (double-repeat) allele of asparagine synthetase as being a risk factor for pancreatitis development (hazard ratio [HR] 8.6, 95% confidence interval [CI] 2 to 37.3) [54]. A study of a multiethnic cohort from the Children's Oncology group and St. Jude identified Native American ancestry as being associated with pancreatitis development, particularly in patients carrying rare nonsense

Table 2
Most notable current pharmacogenomic interactions.

Drug	Most notable pharmacogenomic interactions	Clinical implications; actionability
6-mercaptopurine	TPMT, NUDT15	Increased myelosuppression; preemptive dose decrease indicated
Methotrexate	SLCO1B1	Decreased clearance; avoidance of inhibitors during high-dose therapy
Asparaginase	HLA DRB1*07:01, NFATC2	Increased risk of allergy; no intervention yet available
Vincristine	CEP72	Increased neuropathy; prospective trial of preemptive dose decrease ongoing

HLA = human leukocyte antigen; TMPT = thiopurine methyltransferase.

variants in *CPA2* (HR 587, 95% CI 67 to 5166) [55]. Evaluation of pancreatitis in a Nordic cohort identified rs281366 near *ULK2* as associated with pancreatitis (HR 6.7, 95% CI 3.2 to 14) [56]. Finally, a combined analysis of 10 therapy groups evaluated pancreatitis in a case/control cohort and identified an association with pancreatitis in an expression-quantitative trait locus for trypsinogen (rs13228878 and rs10273639) which was replicated in a Children's Oncology Group cohort [57]. Despite these exciting findings, the inability to replicate these genetic risks consistently across populations suggests further study is needed before altering therapy prospectively due to their presence.

Although thrombosis is a commonly observed toxicity in children and young adults treated with asparaginase, studies have not identified consistent genetic risk factors. Notably, "classic" thrombophilia variants (eg, factor V Leiden, prothrombin G20210A, homozygosity for MTHFR variation) have not been consistently shown to be associated with an increased risk of thrombosis [58-61]. Thus, clinical factors, rather than the presence of prothrombotic variation, appear to be the most consistent drivers of thrombosis risk during ALL therapy.

Vincristine

The vinca alkaloid vincristine blocks microtubule polymerization and is used in multiple phases of ALL therapy, frequently in combination with glucocorticoids. The most frequently encountered toxicity is peripheral motor and/ or sensory neuropathy. This appears to be dose related, with patients receiving higher single and cumulative doses experiencing more neuropathy [62]. Neuropathy also appears to be more common in older vs. younger children, although no differences were observed between adults and children treated with identical therapy [5,62].

Numerous evaluations have been undertaken to identify predisposing genetic factors to vincristine induced neuropathy (VIN). Because vincristine is metabolized by cytochrome P450 3A4 (CYP3A4) and CYP3A5, the literature is rife with reports of increased VIN when used with CYP3A4/5 inhibitors, particularly azole antifungal. Evaluations of CYP3A5 genotype and expression have suggested that polymorphisms which drive higher CYP3A5 expression may be associated with lower rates of VIN [63,64], although that finding has not been replicated in all treatment groups [65].

More recently, GWAS identified an association between a variant (TT at rs924607) in the centrosomal protein 72 (*CEP72*) promoter and the development of VIN in children with ALL [66]. This variant was associated with decreased CEP72 expression, increased sensitivity of nerve cells to disruption by vincristine, and increased in vitro sensitivity of lymphoblasts to vincristine mediated cytotoxicity. The variant allelic frequency is 8% in African Ancestry, 40% in Europeans, and ~30% to 40% in Asian and Hispanic populations. This differential frequency may partly underly lower incidence of VIN in African American populations. Notably, the association is weakened with higher cumulative doses of vincristine, suggesting that the variant lowers the threshold for development of VIN but that increased dose intensity may abrogate the significance of the variant allele. The association between this variant and VIN was also replicated in an adult cohort of ALL patients, in which 75% of patients with the TT genotypes developed VIN compared to 44% of those with either CC or CT genotypes [67]. Prospective genotyping and dose reduction for patients with *CEP72* TT alleles is currently being testing in the St. Jude Total 17 study (NCT03117751).

Glucocorticoids

The glucocorticoids prednisone and dexamethasone are backbone components of all therapies for ALL. Common adverse effects from steroids include hyperglycemia, hyperlipidemia, hypertension, osteopenia, and osteonecrosis [59]. Although these symptoms are typically transient, patients with hyperglycemia are at increased risk of developing type II diabetes as survivors [68]. Leukemia survivors with osteonecrosis also suffer diminished quality of life [69,70].

GWAS have attempted to identify risk variants for osteonecrosis. An analysis of St. Jude Total XV patients identified variants near *ACP* as being associated with the development of osteonecrosis [71]. This variant was also associated with higher lipid levels, a feature associated with an increased risk of osteonecrosis in a separate cohort [72]. Analysis in a pediatric Québécois and Dana-Farber cohort identified an association between *BCL2L11* variants and osteonecrosis development [73].

Work from St. Jude and the Children's Oncology group in contemporary trials identified associations between variants in or near glutamate receptors and increased osteonecrosis risk [74,75]. Genes linked to these variants were also associated with an increased risk of arterial embolism and thrombosis in a large adult cohort. These data suggest that glucocorticoid induced osteonecrosis is driven by vascular factors, a theory supported by both clinical and preclinical data [76-78]. Unfortunately, the variants identified in these studies await validation in external cohorts, with a recent analysis only confirming the association between *ACP* and osteonecrosis in a Dana-Farber/French-Canadian cohort [79]. Based on the available data, it appears that further replication is needed before preemptively intervening to address them.

Conclusions

Pharmacogenomics offers the promise of a future where chemotherapy for ALL is tailored to maximize anti-leukemic efficacy while minimizing therapy toxicity. While the current list of pharmacogenes which are clinically actionable are limited (*TPMT* and *NUDT15*), work is ongoing to assess the effectiveness of interventions on other variants (eg, *CEP72* for vincristine). Moreover, findings from pharmacogenomic studies have elucidated additional mechanisms of disease toxicity which are potentially amenable to targeted interventions. A summary of these are found in Table 2. While many of the associations identified in pediatric studies have been replicated in adults, further study in this population is needed to understand gene/ environment/ age interactions in therapy response and toxicity. Such studies will be needed to unlock the future promised by pharmacogenomics.

Declaration of Competing Interest

The author has no conflicts of interests to disclose.

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