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Abstract:

The field of pharmacogenomics seeks to rationally optimize medication efficacy and minimize adverse effects by correlating genetic variation such as SNPs or gene expression with patient response to the drug therapy. Treatment for most common form of childhood cancer—Acute Lymphoblastic Leukemia—shows great potential for benefitting from pharmacogenomics as many cases experience relapse due to failed drug treatment. Current treatment regimens consist primarily of chemotherapy accompanied with steroids, and if necessary, radiation and stem cell or bone marrow transplants. Chemotherapy is divided into induction and consolidation/intensification states, along with maintenance therapy to ensure all cancerous lymphoblasts have been eradicated. A class of drugs known as thiopurines have been strongly linked to the TPMT pathway; in fact, dosage recommendations have been clinically established for *TPMT* genotypes across populations and treatment protocols (and have been published in PharmGKB/CPIC guidelines). Methotrexate, corticosteroid, and L-asparaginase are other drugs commonly used to treat ALL. Genes found on these pathways such as *GATA3*, *VDR*, *CYP3A5*, *RFC*, *MTHFR*, *TYMS*, *ABCC2*, and *UGT1A1* show strong similar signs of becoming part of a genetic panel for optimizing ALL medication regimens. Although only marginal benefits to treatment can be seen thus far, pharmacogenomics is part of the personalized medicine movement that may one day tailor to all clinical conditions ideally.

### Pharmacogenomics: the “end-ALL” of Acute Lymphoblastic Leukemia?

The advent of high-throughput genomic sequencing has revolutionized personalized medicine. As Felix Frueh, Associate Director of Genomics, FDA puts it, this new field of personalized medicine seeks “[t]he right dose of the right drug for the right indication for the right patient at the right time.” Genomic sequencing has proven to be an invaluable tool for evaluating drug response. Essentially pharmacogenomics seeks to rationally optimize pharmacologic efficacy and minimize adverse effects by correlating genetic variation such as SNPs or gene expression with patient response to the drug therapy. Dr. Russ Altman, principal investigator for the PharmGKB knowledgebase from Stanford, describes, pharmacogenomics is divided into 1) pharmacokinetics (PK: absorption, distribution, metabolism, excretion) and 2) pharmacodynamics and drug response (PD: target, mechanism, response, efficacy, toxicity). “Given the full set of PK genes and PD genes that modulate drug response,” he asks at the Stanford School of Medicine Grand Rounds, “what are the variations in those genes, and how can we personalize dosage in a genome-informed way to optimize this?”

Pharmacogenomics is motivated by the fact that essentially all of the major human drug-metabolizing enzymes exhibit genetic polymorphisms which influence drug metabolism and disposition ([PMID: 12571261](#)). Major genes/encoded proteins that play a role in these drug metabolic pathways include cytochrome P450's enzymes, VKORC1 (Vitamin K epoxide reductase complex subunit 1), and TPMT (Thiopurine methyltransferase). As would be expected from such a bioinformatics endeavor, massive amounts of data govern this new movement, as the genotypes, treatment protocols, and patient information need to be associated to yield meaningful data. But the impacts of implemented pharmacogenomics could be life-saving: from just a simple

blood test, patients can be given dosage regimens tailored to their genetic profiles. The Food and Drug Administration now even requires genetic testing before administering many treatments to prevent adverse reactions.

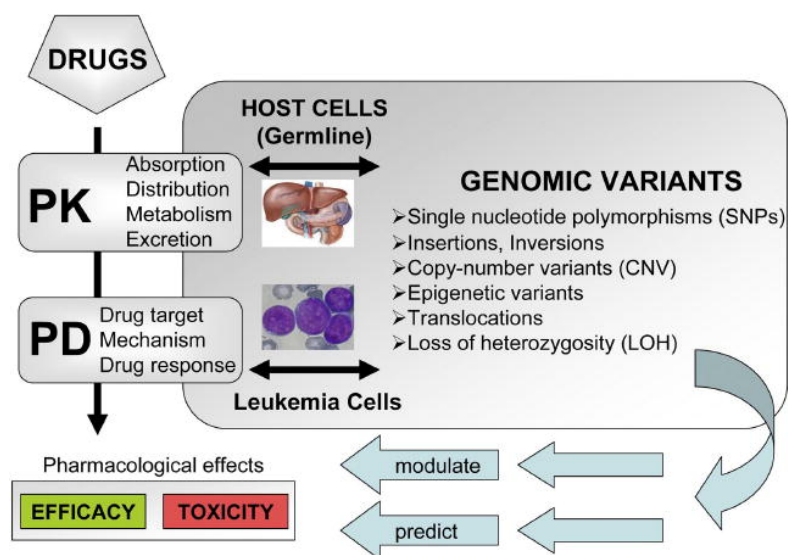


Figure 1—Workflow of Pharmacogenomics ([PMC2665795](#))

One disease that has been the recent focus of pharmacogenomic therapeutic approaches is acute lymphoblastic leukemia (ALL). As its name implies, ALL is a rapidly growing cancer of lymphoblasts, white blood cells found in the bone marrow (“Childhood”). Lymphoblasts are antigen-activated lymphocytes that grow and replicate to ultimately differentiate into Plasma Cells (for B cells), Cytotoxic T cells, and Helper T cells, which are key components of the adaptive immune system (as well as into Natural Killer Cells in the innate system). Cancer arises when these lymphoblasts are overproduced in the bone marrow, which is the site of blood cell production; resources are exhausted by the proliferating cells, causing severe damage via inhibition of normal cell production and infiltration of peripheral blood and organs.

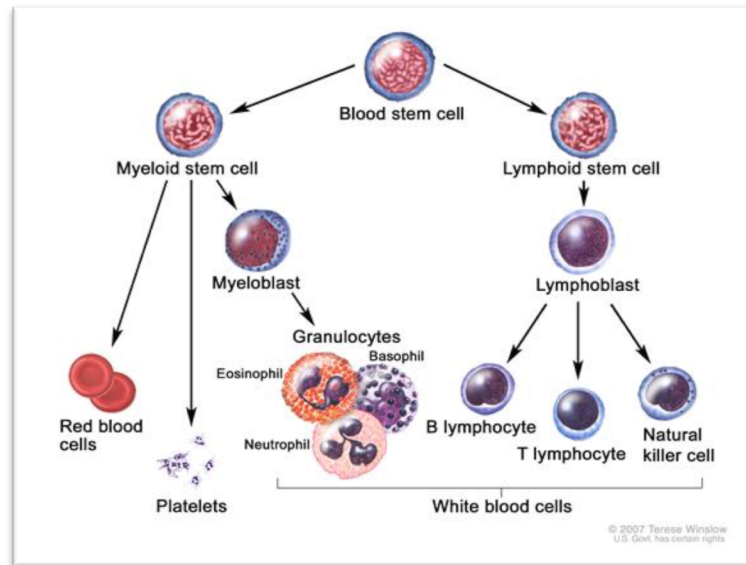


Figure 2 – Human hematopoiesis including lymphoblast differentiation –*TrialX Applied Informatics*

ALL is the most common form of childhood cancer (peak age 2-5 years), accounting for roughly 25% of pediatric malignancies with a population incidence of 1 in 50,000 (marginally higher in males than females, and more common in developed countries) (*American Cancer Society*). Symptoms include bone/joint pain, fever, susceptibility to bruising and bleeding, petechiae, weakness and others (MedlinePlus). Blood tests (CBC, WBC, and Platelet Count) can confirm these symptoms as ALL, along with other diagnostic methods like bone marrow aspiration, biopsy, chest x-ray and spinal tap. Like most cancers, no clear cause is known, but the makeup of this disease suggests that genetic, biological, and environmental factors all play a role in its pathogenesis. Figure 3 shows some of the more common genomic variants associated with ALL. The most common cytogenetic change is a translocation of *TEL* on the short arm of chromosome 12 and *AML1* on the long arm of chromosome 21—the two oncogenes that encode transcription factors are fused—which is associated with a favorable prognosis (Sawinska M., Ladon D 2004). Although perhaps outside the scope of this paper, NCBI's dbVar detected 139

copy number and structural variations in 18 case/control/matched studies, while 41 SNPs were reported in NIH's Catalog of Published Genome-Wide Association Studies (genome.gov). The hope is that some of these variants, or other variants associated with drug metabolic pathways will steer diagnosis through medication.

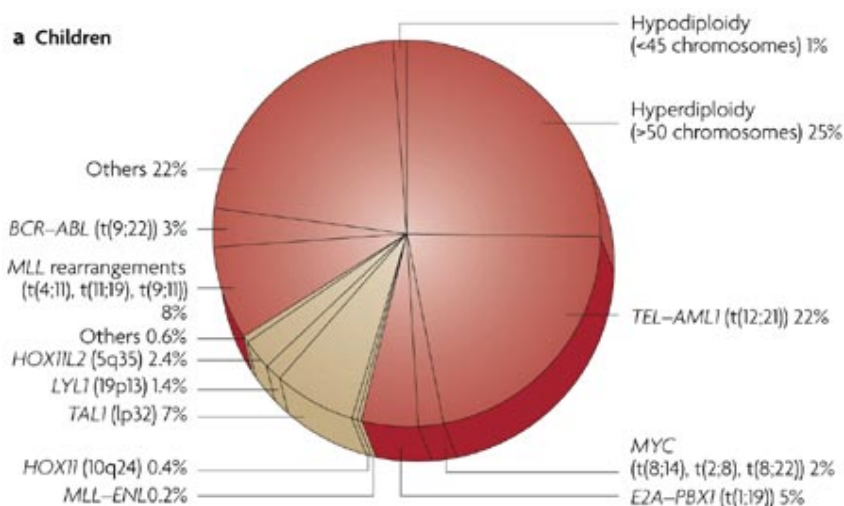


Figure 3-Genetic Variations commonly linked to ALL (*Nature – Drug Discovery*, Pui C. et al. In general, treatment relies heavily on chemotherapy and steroids in conjunction with radiation and stem cell or bone marrow transplants. Although improvements in diagnosis and treatment have led to a 5-year survival rate of 90% and a cure rate of 40% according to the National Cancer Institute, ALL remains the leading cause of cancer-related death in children. Thus, an unmet clinical need exists: ALL's unacceptably high mortality and relapse rate is in large part due to failed treatment from drug resistance.

This paper aims to discuss the panel of treatment options enhanced by the wave of pharmacogenomics, but in order to do so, the current treatment protocol must first be established. While treatment options vary among infants, adolescents, and young adults (with subdivisions according to large chromosomal abnormalities), many commonalities exist in terms of regimens and projected outcomes. Several clinical trials are currently in effect to evaluate the comparative

effects of each treatment (NCT02112916, NCT00526084, NCT00402090, and a few others according to NIH's *ClinicalTrials.gov*). The first step typically consists of remission induction at the initial diagnosis (Ching-Hon Pui et al. 2006). Once complete remission is achieved, stages of consolidation/intensification therapy and maintenance therapy are typically carried out as follow-ups. If these intensive chemotherapy regimens are ineffective however, radiation therapy and immunotherapy along with bone marrow or stem cell transplant to replace lost tissue are usually necessary depending on availability, eligibility, and prognosis.

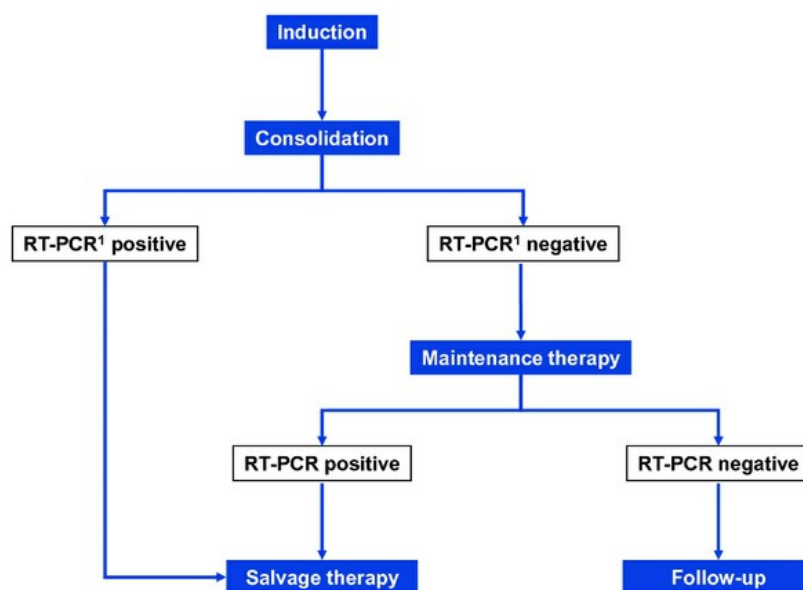


Figure 4—General Algorithm for ALL treatment protocol (*Onkopedia*)

The first stage, remission induction, utilizes the following drugs, with or without an anthracycline: vincristine, corticosteroid (prednisone or dexamethasone), and L-asparaginase. On the Pharmacogenomics Knowledgebase (PharmGKB), only level 3 (not yet replicated) clinical annotations were included on dosage difference between genotypes for “Vincristine;” however, out of the 12 hits, patients with the GG (vs. AG and AA) genotype of *ABCB1*, GG genotype for *ACTG1*, and TT (vs. GG and GT) genotype of *CAPG* have an elevated risk of grade 3-4 (respectively) neurotoxicity. “Prednisone” showed only level 3 evidence as well, with

complications in corticosteroid treatment for pediatric heart transplantations for variations of the gene *ABCB1* and interestingly an efficacy deficiency for AA (vs. AC and CC) genotypes of *GATA3* (this combination chemotherapy study also featured asparaginase, mercaptopurine, methotrexate, dexamethasone, and vincristine). The aforementioned glucocorticosteroids are widely used to treat ALL; these enter cells through passive diffusion, ligand bind to a cytosolic complex, and translocate to the nucleus where they regulate DNA-binding mechanisms, ultimately inducing apoptosis. It is important to note that all of these ALL patients were Caucasian; race cannot be overlooked in pharmacogenomic studies because results are not easily generalized to other situations. Although this *GATA3* result may be a step in the right direction, the molecular basis for glucocorticoid resistance in ALL is still poorly understood. Moreover, L-asparaginase converts aspartic acid to the non-essential amino acid L-asparagine, but in leukemic cells the enzyme asparagine synthetase is absent; the competition from the introduced enzyme thus leads to amino acid starvation and apoptosis in ALL. A pattern of 35 gene expression profiles were differentially identified between resistant and sensitive ALL cells ([PMC2665795](#)). These genes were not statistically significant, but they did point towards a new direction of research: mesenchymal stem cells, which form the microenvironment for lymphoblasts to grow, express asparagine synthetase twenty times higher, which give ALL cells a “safe haven” ([PMID: 17380207](#)). Additionally, Anthracycline is widely studied as a common co-agents in drug treatment, but its pharmacogenomic effects have not been isolated since they are seldom used alone. Furthermore, the predominant PK drug metabolism pathway is via CYP3A, and variations in *VDR* and *CYP3A5* genes (Gene Ontology terms: nuclear hormone receptor, intestinal absorption, drug catabolic process, steroid/small molecule metabolic process, etc.) have been shown to be related to gastrointestinal toxicity. These relations must be associated in more

clinical contexts to confirm validity because the slightest variations in medication protocol can lead to false correlations. Complete remission is achieved in 98% of newly diagnosed B-precursor ALL from this first treatment onslaught, but this number is significantly lower in more developed ALL cases.

The true potential for implementing pharmacogenomics in ALL treatment occurs in this second stage. Intensification/consolidation chemotherapy is next carried out according to the *Berlin-Frankfurt-Münster (BFM) Backbone* ([PMID: 20010625](#)), described here:

1. An initial consolidation (sometimes referred to as “Induction IB”) immediately after the initial induction phase. This phase includes cyclophosphamide, low-dose cytarabine, and a thiopurine (mercaptopurine or thioguanine).
2. An interim maintenance phase, which includes multiple doses of either intermediate-dose or high-dose methotrexate ( $1\text{--}5\text{ g/m}^2$ ) with leucovorin rescue or escalating doses of methotrexate (starting dose  $100\text{ mg/m}^2$ ) without leucovorin rescue.
3. Reinduction (or delayed intensification), which typically includes the same agents used during the induction and initial consolidation phases.

Since leucovorin, cyclophosphamide, and cytarabine are complementary medications, this discussion will focus on the primary agents of thiopurines and methotrexate. Thiopurines mercaptopurine and thioguanine are analogues of purine nucleosides hypoxanthine and guanine. Following uptake via nucleoside transporters, these drugs are catabolized into active cytotoxic thioguanine nucleotides (TGNs). When TGNs are incorporated into RNA or DNA, cell cycle arrest occurs leading to apoptosis. A ubiquitously cytosolic enzyme known as TPMT (GO terms methylation, small molecule metabolic process) catalyzes the S-methylation of thiopurines; this is relevant to ALL because the TPMT pathway found in hematopoietic tissues seeks a balance between TGNs and inactive metabolites, with TPMT acting as the regulator (i.e. without a normal functioning TPMT allele, patients are at a high risk for blood toxicity as a result of thiopurine therapy). As expected PharmGKb overflowed with much stronger hits. Since 2011 (evidence through 2013 confirmed past findings), a well-founded dosage protocol has been



established for *TPMT* genotypes. Published in *Clinical Pharmacology and Therapeutics*, the full dosing recommendations can be found here: [Clinical Pharmacogenetics Implementation Consortium Guidelines for TPMT Genotype and Thiopurine Dosing: 2013 Update](#). In summary, individuals with homozygous deficient alleles exhibit severe myelosuppression; thus, a drastically lower dose of mercaptopurine, thioguanine, or azathioprine are recommended than those with normal diplotypes. The activity levels vary greatly across ethnicities, but many supplemental tables of information have been published to aid clinicians in the apt dosages for ALL patients.

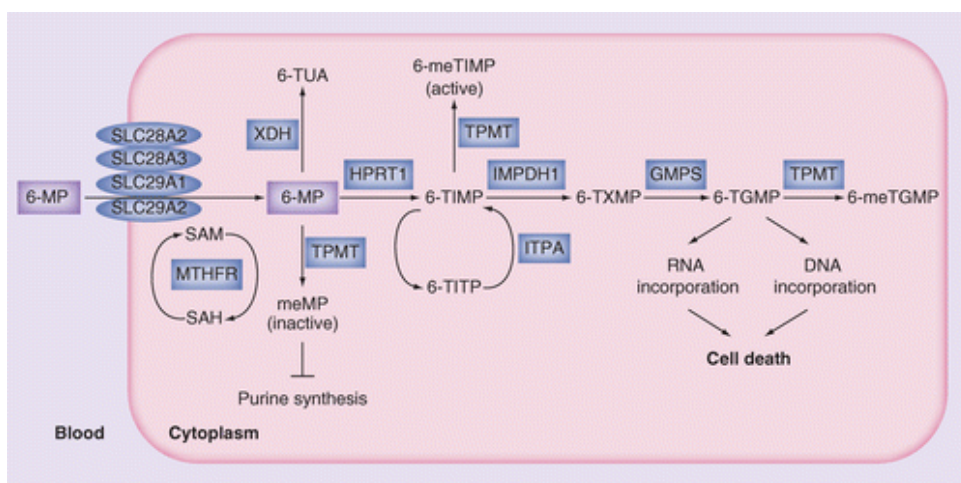


Figure 5—First Main Drug Pathway Relevant to ALL— Thiopurines (Lopez-Lopez)

Methotrexate has also been a focus of pharmacogenomic optimization ([PMID: 23652803](#)). *RFC* (*SLC19A1*) (GO Terms: Folic acid metabolic process, transporter activity) mediates the uptake of methotrexate, and once inside the leukemic cells, it is converted to long chain forms which accumulate and disrupt DNA synthesis, inducing necrosis. AA or AG genotypes of *RFC* have typically predicted gastrointestinal toxicity. Currently, high dosages of antifolate methotrexate are given universally as consolidation therapy (and low dosages in childhood continuation therapy); however, optimal dosages have yet to be determined as the pharmacokinetics understanding is incomplete. PharmGKB only detects one clinical annotation

relating to toxicity in ALL patients (evidence level 1B). Individuals of most populations (varying ethnic cohorts) with genotype of AA in *MTHFR* (GO terms: tetrahydrofolate conversion, response to folic acid, blood circulation, etc.) have an increased risk of oral mucositis.

Thymidylate synthase (encoded by *TYMS*) is a target of folate-dependent enzymes, and the transporter protein from the *ABCC2* gene also is key to excreting methotrexate. Variants of these two genes may also provide genomic insight into the basis of inter-patient differences of methotrexate response.

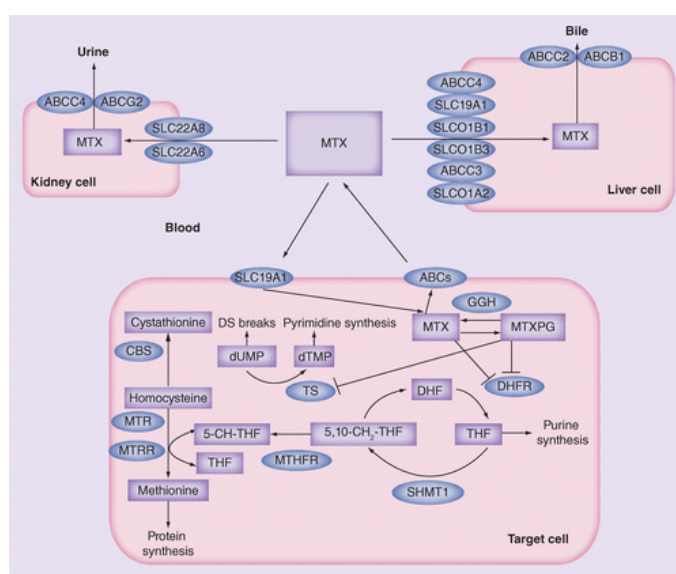


Figure 6—Second Main Drug Pathways Relevant to ALL—Methotrexate (Lopez-Lopez)

Lastly, in the induction, consolidation, and continuation phases, a promoter repeat polymorphism in *UGT1A1* has consistently predicted hyperbilirubinemia and jaundice ([PMC2665795](#)). The third stage, maintenance therapy, includes daily oral mercaptopurine, weekly oral or parenteral methotrexate, and sometimes vincristine/steroid pulses as the backbone in most protocols; the same reagents have been discussed above, so the pharmacogenomic analysis is equivalent. However, this stage is the most critical for apt dosages as failed drug treatment may lead to relapse (which currently is the largest problem in treating ALL).

Despite the large number of targets on these drug pathways, as of 2014, *TPMT* is the only ALL pharmacogenetic marker with clinical guidelines for drug dosing due to its well understood mechanism. Pharmacogenomics aims to expand this to a whole genetic profile (including not only exome data, but also noncoding and epigenetic variations among others), whereby genotypes can guide a panel of quantitative dosage regimens for truly personalized medicine. Much more clinical trials across various populations and treatment protocols will be needed to ensure reliable pharmacogenomic guidance.

Pharmacogenomics is a field that operates on very small margins of treatment improvement. Only in certain cases would a genetically directed treatment be noticeably better than the one-size-fits all treatment. Some may question whether the marginal benefit is worth the extra resources necessary for research and genetic testing. But when we are discussing thousands of children with promising lives ahead that are suffering from Acute Lymphoblastic Leukemia, even in the remaining 20% that fail to be cured, the cost is irrelevant. ALL is a well-studied cancer that can be optimized further by pharmacogenomic techniques. A simple blood test for genotypes of *TPMT*, *GATA3*, *VDR*, *CYP3A5*, *RFC*, *MTHFR*, *TYMS*, *ABCC2*, and *UGT1A1* (once these genetic mechanism have been validated and generalized across populations at a clinical level) could markedly improve dosage regimens for ALL victims. For instance, this could be especially critical for later stage ALL patients, who must endure radical treatment to eradicate cancerous cells while salvaging as much tissue as possible. Even in less severe cases, knowing all the side effects could be the difference between life and death. So while pharmacogenomic developments may not be the “end-ALL” of Acute Lymphoblastic Leukemia just yet, personalized medicine techniques surely seem to be moving closer to optimized treatment for *all* diseases.

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