THIOPURINE METABOLISM AND THE RESPONSE TO MAINTENANCE THERAPY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) treatment comprehends three main phases: induction, intensification/consolidation, and maintenance therapy (MT), which includes two oral drugs, 6-mercaptopurine (6-MP) and methotrexate. MT is an essential phase in ALL treatment being associated with lower incidence of relapse. However, there is no consensus about the parameters for drug monitoring and dose adjustments, due to great interindividual variations in bioavailability and pharmacokinetics of 6-MP. Thiopurine S-methyltransferase (*TPMT*) is the main pharmacogene associated with response to 6-MP, being the variant alleles **2, *3A, *3B* and **3C* related to increased toxicity (Figure 1). Therefore, our aim is to investigate the effect of genetic variations of 6-MP metabolism on ALL treatment response in a Brazilian cohort.

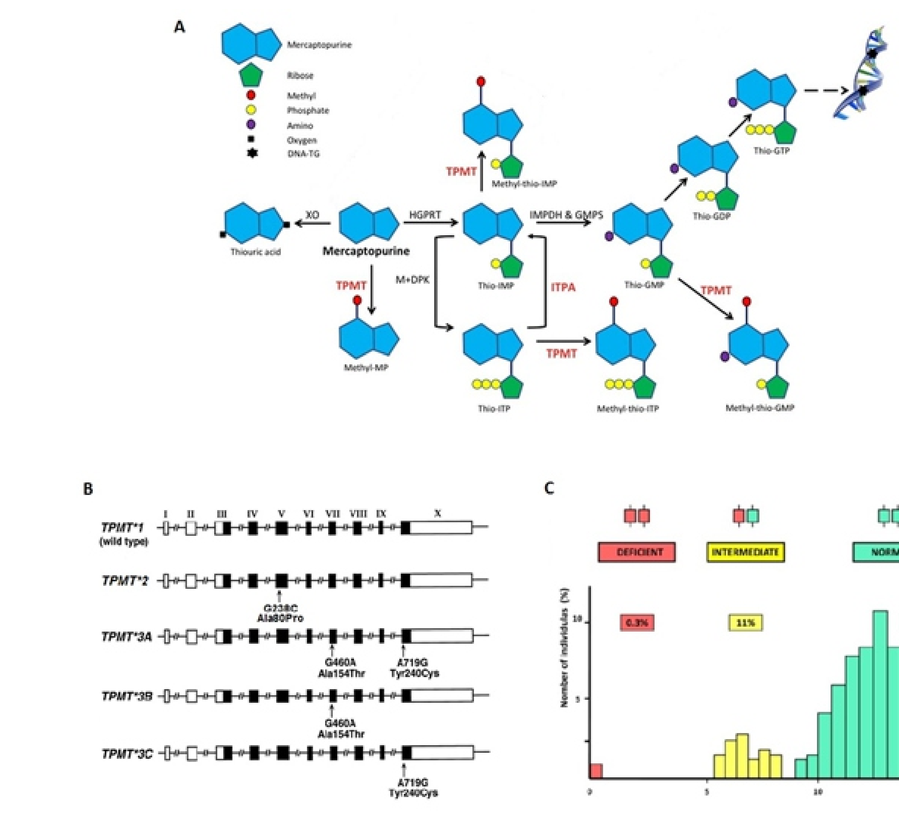

Figure 1. 6-MP metabolism and *TPMT* polymorphisms. (A) 6-MP has 3 major metabolic pathways. First, a fraction of 6-MP is converted to inactive 6-thiouric acid by the enzyme xanthine oxidase in first pass metabolism. Second, 6-MP is a prodrug that through a multistep process is converted to 6-TGN, which, in a deoxy form, is incorporated into DNA in nucleated cells. A third metabolic pathway is thiomethylation of 6-MP and some of its metabolites catalyzed by TPMT, thus reducing 6-TGN formation. 6-MP, 6-mercaptopurine; 6-TGN, 6-thioguanine nucleotide. DNA-TG, thioguanine nucleotides incorporated into DNA; GMPS, guanosine monophosphate synthetase; HGPRT, hypoxanthine guanine phosphoribosyl transferase; IMPDH, inosine monophosphate dehydrogenase; ITPA, inosine triphosphate pyrophosphatases; M+DPK, monophosphate and diphosphate kinases; TPMT, thiopurine methyltransferase; XO, xanthine oxidase. Adapted from SCHMIEGELOW et al, 2014. (B) The variant alleles *TPMT*2 (*rs1800462), *TPMT*3B* (rs1800460), *TPMT*3C* (rs1142345) and *TPMT*3A* (both *TPMT*3B* and *TPMT*3C*) result in reduced enzymatic activity. Adapted from WANG et al, 2010. (C) Genotype-phenotype correlations and trimodal distribution of TPMT activity. Adapted from WOILLARD et al, 2017.

MATERIAL AND METHODS

This study will be held in two phases (Figure 2). The first phase comprehends a retrospective analysis of the associations of *TPMT* genotypes with treatment outcomes, including approximately 600 ALL cases aged up to 19 years old, diagnosed between 2012 and 2017. The second phase will prospectively include approximately 300 ALL cases diagnosed between 2018 and 2020, in which *TPMT* genotypes will be associated with the amount of thioguanine incorporated to deoxyribonucleic acid (DNA-TGN) in peripheral leukocytes during MT. DNA will be purified from peripheral blood samples using QIAamp DNA Blood Mini Kit. TPMT polymorphisms *(*2, *3A, *3B* and **3C* alleles) will be identified by TaqMan® SNP Genotyping Assay (Figure 3). For DNA-TGN quantification, 1-2 µg of DNA will be purified from whole blood, depurinised, ethenoderivatised with chloroacetaldehyde, and measured for guanine and thioguanine content by ultra performance liquid chromatography - tandem mass spectrometer (JACOBSEN et al, 2012). For that, samples of peripheral blood will be collected in three distinct time points during MT. Demographic and clinical data (Box 1) will be collected from standardized electronic questionnaires. Outcomes were defined as myelotoxicity, 6-MP dose adjustment, relapse occurrence, and death. Genotypic frequencies of *TPMT* polymorphisms will be compared between groups with different outcomes by chi-squared or Fisher's test. Risk associations will be estimated by hazard ratios with confidence interval of 95%. Overall survival and event free survival will be estimated by Kaplan Meier, and groups will be compared by log-rank test. Statistical analysis will be held with SPSS Statistics 18.

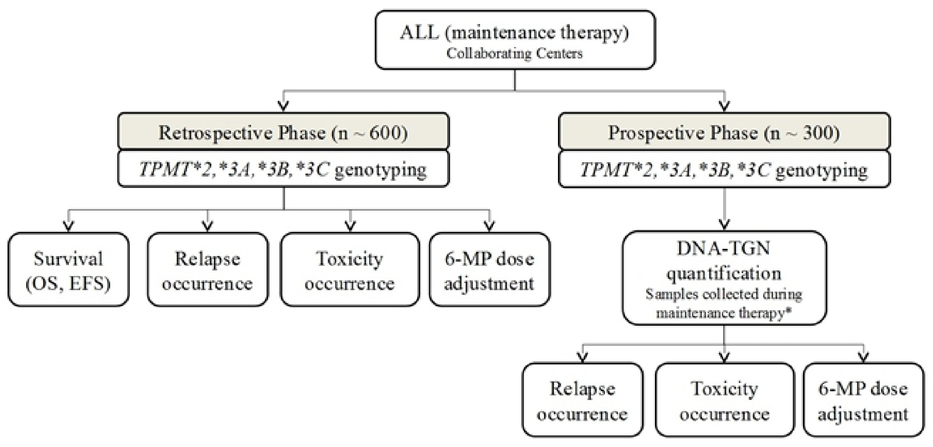

Figure 2. Study design. ALL, acute lymphoblastic leukemia. OS, overall survival. EFS, event-free survival. 6-MP, 6-mercaptopurine; DNA-TG, thioguanine nucleotides incorporated into DNA. *Peripheral blood samples will be collected in three distinct time points during ALL treatment: before initiating maintenance therapy (D0), one week after initiating maintenance therapy (D8), and three months after initiating maintenance therapy.

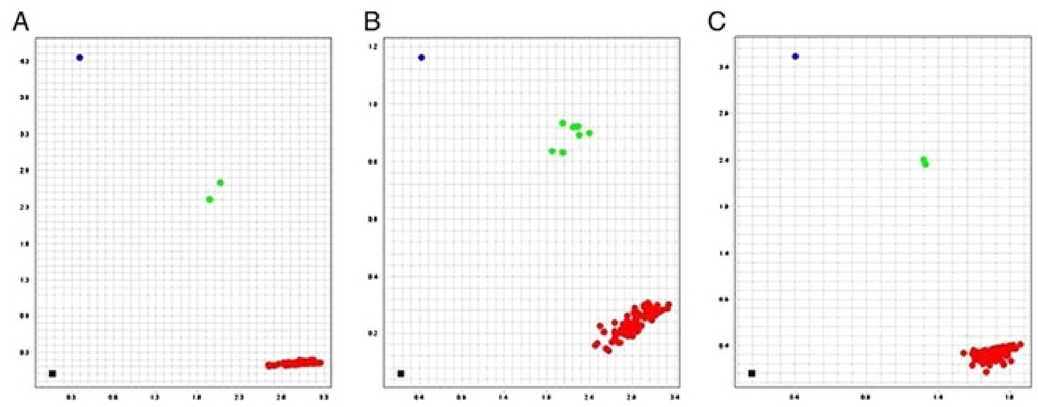

Figure 3. Representative allelic discrimitation results for TaqMan genotyping assays *TPMT*2* (A), *TPMT*3B* (B), and *TPMT*3C* (C). Red dots, wild-type genotype; green dot, heterozygous genotype; blue dot, homozygous variant genotype; black square, no template control. (BUCHARD et al, 2014)

Box 1. Demographic and clinical data that will be collected from standardized questionnaires.

At diagnosis	Follow up
Age, sex, skin color	Minimal residual disease (MRD) D29-35
White blood cell count	Occurrence of toxicity events (myelotoxicity, need for 6-MP dose adjustments)
Risk group (according to NCI)	Relapse occurrence
Molecular-cytogenetic alteration	Vital status

PERSPECTIVES

Although it is consensual that MT is essential to ALL treatment outcome, there is still a need for identifying a good marker for drug monitoring of 6-MP. With this study, we expect to contribute to scientific knowledge about pharmacogenetics of ALL treatment, especially regarding the role of TPMT genotypes on treatment outcomes in the Brazilian population, and the use of DNA-TGN measurement for drug monitoring during MT.

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