



## **STUDY PROTOCOL**

### **Haematotoxicity in Maintenance Therapy of Children With Acute Lymphoblastic Leukemia: Focus on Genotyping and Phenotyping of Mercaptopurine**

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## Background

Leukemia is the most common malignant disease in children, of 30% of children under 15 years diagnosed with cancers caused by leukemia. In children with leukemia, 80% have acute lymphoblastic leukemia (ALL). ALL The highest cause of death in children with cancer.

The success rate of LLA patients reaches a remission of more than 90%, but 30-40% of them relapse in the maintenance phase.<sup>5</sup> In developed countries, the last few years, the incidence of relapse to 11% .<sup>6</sup> Meanwhile, the relapse rate in RSCM is still 28 , 7% .<sup>7</sup> To achieve long-term remission, LLA patients require maintenance phase therapy for 2-3 years with mercaptopurin daily and low-dose methotrexate once a week.<sup>8</sup> Drug toxicity during the maintenance phase is one of the main concerns not only because Can be life-threatening, but is a major cause of treatment discontinuation, which can increase the risk of relapse

During the maintenance phase, patients received 6-mercaptopurine therapy orally daily and low dose methotrexate week sekali. Mercaptopurine (6MP) is the main drug in this phase. The efficacy and toxicity of 6MP affected by enzyme activity TPMT.<sup>21,22</sup> TPMT enzyme activity is influenced by the presence of genetic polymorphisms autosomal co-dominant populations variability etnik.<sup>23-25</sup> existence of the gene encoding the enzyme permetabolisme medications will cause drug response differences between individuals, Which may affect treatment outcomes.<sup>26</sup> To date there are 30 known variations of TPMT alleles.<sup>27</sup> Wild type alleles, TPMT \* 1, show full enzyme activity. TPMT \* 2, \* 3A, \* 3B and \* 3C alleles are polymorphic alleles that are found in caucasian populations. All four variations of the allele showed decreased TPMT enzyme activity.<sup>23-25,27</sup> Based on population studies, the distribution of TPMT enzyme activity in erythrocytes was trimodal; The majority of the population (89-94%) had a homozygous wild type allele exhibiting high TPMT activity, 6-11% had heterozygous mutant alleles with moderate TPMT activity, and 0.3% had homozygous mutant alleles showing low or undetectable TPMT activity.

Variations in TPMT enzyme activity due to polymorphism are the most important factors affecting intracellular 6TGN accumulation after 6MP administration.<sup>32</sup> TPMT activity is inversely proportional to 6TGN concentrations in erythrocytes in LLA child patients.<sup>28,33</sup> In homozygous wild type patients, their erythrocyte 6TGN levels are lower Compared with heterozygote mutant patients.<sup>34,35</sup> Homozygous mutant patients had the highest 6TGN levels.<sup>35</sup> Compared with homozygous wild type patients, heterozygous mutant patients required a lower 6MP dose, 30-50% of the standard dose, 22,31,36 but at risk Experienced severe myelosuppression.<sup>37</sup> In homozygous mutant patients, the 6MP dose was reduced to 10-15 times less than the recommended dose, 28,37,38 because of the excessive accumulation of 6TGN in hematopoietic tissue, which may have life-threatening side-effects of myelosuppression.

The frequency and pattern of TPMT mutant alleles differ among different ethnic populations. In the American-Caucasian and African-American populations, all three mutant TPMT \* 2, \* 3A, \* 3C mutants are found, but the most mutant alleles in the US-Caucasian allele are TPMT \* 3A (3.2%), and in African-Americans TPMT \* 3C (2.4%). In the Southwest Asian (India and Pakistan), only 1% of the mutant alleles are TPMT \* 3A. In the Southeast Asian population, only 1% TPMT \* 3C mutant alleles were found. Similarly in populations in Thailand, Japan, and China, only TPMT \* 3C mutant alleles are found at 1-4.74%.<sup>23,24,27,31</sup> The frequency and pattern of TPMT mutant alleles in the population of children with LLA in Indonesia is currently unknown

**Study Objectives:**

1. Knowing the distribution of TPMT \* 3A and \* 3C genotypes, 6TGN / 6meMP erythrocyte level, risk stratification, nutritional status, and albumin levels in LLA child patients in Indonesia.
2. Analyzing the association of hematotoxicity with TPMT \* 3A and \* 3C genotypes, erythrocyte 6TGN / 6meMP ratio, risk stratification, nutritional status, and albumin levels in the LLA child population in Indonesia.
3. Analyze the genotype associations of TPMT \* 3A and \* 3C with 6TGN / 6meMP erythrocyte ratio in LLA child.

**Study Design:**

This study used an observational, cross sectional study to obtain data on patient demographics, nutritional status, albumin levels, TPMT genotype, and 6TGN / 6meMP levels in LLA children.

## Statistical Analysis Plan (SAP)

Data is presented in the form of narration, tables or graphs. Subject characteristics data, TPMT polymorphism frequency, hematology effect, TGN and 6-meMP concentration, and TGN/6-meMP ratio in erythrocytes are presented descriptively, in mean (standard deviation), or median value (range of values) And in percentages.

To assess the relationship of TPMT polymorphism with 6TGN/6meMP ratio in erythrocytes using unpaired t test (normal distribution) or Mann Withney (abnormal distribution):

Genotype	Ratio TGN/6meMP (mean)		p-Value
	Wild type	Mutant	

To assess the association of hematologic toxicity with factors that may affect it:

	Variables	Hematotoxicity		p-value	OR	95% CI
		+	-			
		(Grade 1–4)	(Grade 0)			
<b>Genotype<sup>a</sup></b>	- Wild type - Mutan					
<b>Phenotype<sup>b</sup></b>	6TGN/6meMP Ratio					
<b>Risk</b>	- High risk					
<b>Stratification<sup>a</sup></b>	- Standard risk					
<b>Nutritional Status<sup>a</sup></b>	- poor - normal-over weight					
<b>Albumin levels<sup>a</sup></b>	- Hypo - Normal					

OR= *odd ratio*; CI= confidents interval

<sup>a</sup> Statistical method:  $\chi^2$  test or Fischer test

<sup>b</sup> Statistical method: unpaired t test (normal distribution) or Mann Withney (abnormal distribution):

## **Informed Consent**

We, a team of researchers at the Pharmacology and Therapeutic Section of the Faculty of Medicine, University of Indonesia, Jakarta, are conducting a study to determine the incidence of blood toxicity in pediatric patients with acute lymphoblastic leukemia (LLA) currently in maintenance phase therapy, and associated with mercaptopurin drug Routine given by the doctor during maintenance phase therapy). This study does not increase or decrease the drugs given to you. Abroad, the dosage of mercaptopurine given is adjusted to the patient's genotype, to avoid the incidence of blood toxicity in the patient. In Indonesia, this dose adjustment has not been performed, as there has been no research on genotypic data in LLA child patients in Indonesia. This study will include one hundred LLA patients aged 1-18 years. You / your child have LLA and are therefore asked to participate in this research.

If you are willing to participate, during routine blood tests, your doctor will take your blood sample as much as one tablespoon of blood vessels in your arm by using a syringe. Blood-collection of arm arms usually causes only mild pain, but sometimes infections and / or swelling and blue, which will heal after a few days of treatment. Blood sampling for this study was performed only once. You are exempted from the routine blood examination fee, and you get a replacement transport of one hundred thousand rupiah (Rp 100.000, -). In addition, your doctor will take data from your medical record. This study is of no direct use to you, but the results are useful for determining whether or not genotypes are needed to adjust the mercaptopurin dosage to avoid the incidence of blood toxicity in LLA patients. You are free to refuse to participate in this research without any adverse effects. If you have decided to participate, then you are also free to resign at any time without causing any change in the quality of service from your doctor.

All of this research data will be treated in secret so as not to allow others to connect with you. You are given the opportunity to ask all the things that are not clear with regard to this research. If at any time you need an explanation, you can contact Doctor Dewi Selvina Rosdiana in Pharmacology and trapeutic Section of Faculty of Medicine University of Indonesia, Jl. Salemba Raya no.6, Central Jakarta, no phone 089517704616.

### **Approval Form**

All of the above explanations have been submitted to me and all my questions have been answered by the doctor. I understand that if I still need an explanation, I will get an answer from Dr. Dewi Selvina Rosdiana. By signing this form, I agree to participate in this research.

**Patient's signature**

**Date:**

**Patient's Name:** \_\_\_\_\_

**Witness signature**

**Name of witness:** \_\_\_\_\_

**Investigator's signature:**

**Investigator's Name:** \_\_\_\_\_