Biomarkers for gastrointestinal adverse events related to thiopurine therapy

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Author contributions: Zudeh G and Franca R wrote, revised, and edited the manuscript; Stocco G conceptualized, edited, and revised the manuscript; Decorti G conceptualized, wrote, revised, edited, and supervised the manuscript; all authors read and approved the final manuscript.

Conflict-of-interest statement: All authors declare no conflicts of interest for this manuscript.

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Abstract

Thiopurines are immunomodulators used in the treatment of acute lymphoblastic leukemia and inflammatory bowel diseases. Adverse reactions to these agents are one of the main causes of treatment discontinuation or interruption. Myelosuppression is the most frequent adverse effect; however, approximately 5%-20% of patients develop gastrointestinal toxicity. The identification of biomarkers able to prevent and/or monitor these adverse reactions would be useful for clinicians for the proactive management of long-term thiopurine therapy. In this editorial, we discuss evidence supporting the use of PACSN2, RAC1, and ITPA genes, in addition to TPMT and NUDT15, as possible biomarkers for thiopurine-related gastrointestinal toxicity.

Key Words: Thiopurines; Gastrointestinal adverse effects; Biomarkers; PACSN2; RAC1; ITPA

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Core Tip: Adverse reactions to thiopurines are one of the main causes of treatment discontinuation or interruption. In addition to myelosuppression, approximately 5–20% of patients develop gastrointestinal toxicity; the identification of biomarkers to prevent and/or monitor these adverse reactions is important for the proactive management of long-term thiopurine therapy. In this editorial, we discuss evidence supporting the use of PACSN2, RAC1, and ITPA genes, in addition to TPMT and NUDT15, as possible...
Mechanisms of action and adverse effects of thiopurine

Thiopurines, such as mercaptopurine (MP) and its prodrug azathioprine (AZA), are immunomodulatory drugs used in the treatment of pediatric acute lymphoblastic leukemia (ALL) and nonmalignant conditions, such as inflammatory bowel diseases (IBDs)[1,2]. These immunomodulators undergo a complex biotransformation that leads to the production of different thionucleotides (TGNs), such as thioguanosine mono-, di-, and triphosphate (tGMP, tGDP, and tGTP) and deoxythioguanosine mono-, di-, and triphosphate (tdGMP, tdGDP, and tdGTP) (Figure 1). These purine antimetabolites exert their cytotoxic activity through different mechanisms, such as inhibition of de novo purine synthesis, interference with the incorporation of guanosine nucleotides into DNA and RNA, and induction of apoptosis due to inhibition of the Ras-related C3 botulinum toxin substrate 1 (Rac-1) protein, a Rho-GTPase[3]. Under physiological conditions, Rac-1-GTP activates the MEKK/IκB/NF-κB and STAT3 survival pathways in activated lymphocytes, resulting in an increase in the antiapoptotic protein Bcl-xL, whereas during thiopurine treatment, the binding of tGTP to Rac-1 impairs these pathways, enhancing apoptosis[3]. Thiopurines are also processed through catabolic pathways, in which xanthine oxidase and thiopurine methyltransferase (TPMT) are the main enzymes involved, producing inactive metabolites such as thiouric acid and methylmercaptopurine, respectively. TPMT also catalyzes the S-methylation of intermediates resulting from MP conversion to TGN, leading to the production of secondary methylated nucleotides (MMPNs) (Figure 1). The role of MMPN metabolites is not fully characterized; however, they could contribute to the inhibition of de novo purine synthesis. Factors affecting the TGN/MMPN ratio could influence thiopurine efficacy and toxicity. For example, the amount of TGN in white blood cells is responsible for the immunosuppressive effects; when TPMT activity is compromised, TGN levels increase, leading to dangerous myelosuppression[3].

Thiopurines have a narrow therapeutic index, with an increased risk of severe toxicity and treatment discontinuation[4]. Direct cytotoxic damage can occur in proliferating cells of different tissues and organs. In particular, thiopurines have been associated with the dose-dependent hematological toxicity observed in approximately 80% of ALL cases; in IBD patients, the incidence of bone marrow toxicity is lower (approximately 10%)[5,6]. Neutropenia and leukopenia are the most frequent outcomes of myelosuppression, related to an increased risk of infection, and the main reasons for therapy discontinuation or interruption that can lead to disease aggravation in both ALL and IBD[7-9]. Thiopurine-induced gastrointestinal (GI) toxicity occurs in approximately 5%-20% of ALL and IBD patients; the main symptoms are nausea, vomiting, stomatitis, abdominal pain or cramping, gastritis, gastric ulcer, GI bleeding, and diarrhea[10,11]. Moreover, these immunosuppressors are associated with the risk of neurological complications, hepatotoxicity, pancreatitis, arthralgia, and skin rash[10,12-16].

In the clinic, white blood cell counting is commonly performed to monitor the immunosuppressive effects of these drugs; however, recently, pharmacogenetic biomarkers for predicting thiopurine-induced hematological adverse events have been identified. From a pharmacogenetic point of view, TPMT is one of the best characterized genes[17]. Both TPMT protein expression and enzymatic activity are affected by the presence of variants in the TPMT gene. More than 44 TPMT variant alleles have been described; TPMT*2 (rs1800462, 238G>C, p.Ala80Pro), TPMT*3B (rs1800460, 460G>A, p. Ala154Thr), TPMT*3C (rs1142345, 719A>G, p. Tyr240Cys), and TPMT*3A (rs1800460 and rs1142345 haplotypes) are the most frequent variants in Europeans and can explain up to 95% of TPMT deficiencies[18-20]. As reported above, decreased TPMT activity leads to higher TGN levels and lower MMPN in white blood cells; these
BIOMARKERS FOR THIOPURINE-INDUCED GASTROINTESTINAL ADVERSE EVENTS

Although genome-wide association studies (GWAS) have indicated that TPMT activity is predominantly a monogenic trait[29], a percentage of wild-type TPMT carriers present reduced TPMT activity, suggesting the existence of other regulatory mechanisms able to modulate its function[30,31]. In 2012, Stocco et al[32] demonstrated...
that the expression levels and the single nucleotide polymorphism (SNP) rs2413739 of the protein kinase C and casein kinase substrate in neurons 2 (PACSIN2) gene were associated with TPMT activity in HapMap cell lines and in a cohort of ALL pediatric patients enrolled at St. Jude Research Children Hospital (SJRH, Memphis, United States), suggesting a possible role of PACSIN2 as a TPMT modulator[32]. The authors found that the intronic variant rs2413739 (C>T) was associated with an increased risk of severe GI toxicity during consolidation therapy in two independent cohorts of ALL pediatric patients treated according to the SJRH Total 13B protocol and to the Associazione Italiana Ematologia Oncologia Pediatrica/Berlin-Frankfurt-Münster (AIEOP-BFM) 2000 protocol[32]. Patients received 75 mg/m² MP daily and 2 g/m² high-dose methotrexate (HD-MTX) i.v. twice a week for 2 wk at SJRH, whereas those undergoing the AIEOP-BFM 2000 protocol were treated daily with 25 mg/m² MP and received four HD-MTX (2-5 g/m²) infusions once every 2 wk. To further validate these results, Franca et al[33] investigated the possible role of PACSIN2 rs2413739 in an additional cohort of ALL pediatric patients treated according to the AIEOP-BFM 2009 protocol, with the same consolidation phase as AIEOP-BFM ALL 2000, and in a cohort of IBD pediatric patients undergoing AZA therapy. In the ALL cohort, the PACSIN2 T allele was associated with decreased TPMT activity during maintenance therapy, particularly in patients heterozygous for TPMT rs1142345 and rs1800460. Moreover, the PACSIN2 TT genotype was associated with a higher risk of GI toxicity during the consolidation phase. The latter association was borderline, likely because of the limited number of clinical data available (n = 81); however, it was in line with the findings of Stocco et al[32]. Far more complex to understand is thiopurine-induced GI toxicities in IBD patients, where the occurrence of adverse effects overlaps with clinical manifestations of the disease. Interestingly, Franca et al[33] showed that IBD patients carrying the PACSIN2 T allele and undergoing AZA treatment presented a more active disease, measured as pediatric ulcerative colitis activity/pediatric Crohn’s disease activity (PUCAI/PCDAI) indices > 10, according to standard clinical practice. No association between the rs2413739 variant and either TPMT activity or TGN/MMPN levels was found, suggesting a thiopurine-independent effect on the clinical phenotype[33]. Enzymatic activity was significantly higher in the ALL patients than in the IBD patients[33]. The different impact of PACSIN2 SNP rs2413739 on TPMT activity could be partially explained by patient age: The ALL cohort comprised children under 10 years, while the IBD patients were mainly teenagers. The authors hypothesized that the PACSIN2 genetic impact on TPMT activity could be more evident in younger patients, who seemed to have increased TPMT activity[34,35]. Moreover, concomitant treatment with MTX in the ALL cohort could contribute to discrepancies in the results; MTX could impact S-adenosyl methionine levels, a TPMT cofactor responsible for the stability of the protein[36]. Since Franca et al[33] did not detect significant changes in TGN levels in PACSIN2 T allele carriers, they hypothesized a thiopurine-independent effect of PACSIN2 on GI toxicity and a tissue-specific role of PACSIN2 in the intestine. Notably, the Genotype-Tissue Expression Portal (GTEx) shows that PACSIN2 and TPMT expression levels are increased in blood and in the esophageal mucosa of healthy PACSIN2 rs2413739 T allele carriers but not in the small intestine and colon of these subjects, supporting the idea that the enhanced GI toxicity observed in TT patients is not related to differential expression of TPMT in the GI tract[37]. Other evidence regarding PACSIN2 suggests its role as a regulator of intestinal mucosal homeostasis and inflammation. Intriguingly, an underinvestigated mechanism of IBD pathogenesis is VE-cadherin-directed vascular barrier disruption[38], and PACSIN2 has been recognized as a regulator of cell-cell adhesion in the endothelium through the inhibition of asymmetric VE-cadherin internalization from adherens junctions[39]. Stocco et al[32] performed an agnostic gene expression analysis in the human B leukemia cell line NALM6 and identified autophagy as one of the pathways significantly affected by PACSIN2 knockdown, thus suggesting a possible role of this gene in autophagy, another mechanism involved in IBD pathogenesis[32,40,41]. Moreover, the human protein ATLAS report shows that lower levels of PACSIN2 are related to a reduced survival probability in colorectal adenocarcinoma patients, leaving open the question of whether PACSIN2 is a marker of therapeutic response or a contributing factor to intestinal cancer progression[42]. Dedicated studies to clarify the issue of PACSIN2 and GI pathology are needed; however, all this evidence supports the hypothesis that PACSIN2 could be a susceptibility factor for intestinal tissue damage.

Thiopurine-derived tGTPs are able to compete with GTP on Rac-1, a Rho-GTPase involved in cellular proliferation. It can be hypothesized that factors reducing Rac-1 expression or activity could influence cell susceptibility to cytotoxic stimuli, thus contributing to thiopurine efficacy and toxicity. Interestingly, Rac-1 was able to bind
PACSIN2 through a physical interaction[3]; this protein–protein interaction seemed to be responsible for reciprocal regulation: Rac-1 activity controlled PACSIN2 cellular distribution, whereas PACSIN2 could negatively modulate Rac-1 activity[43]. In vitro data showed decreased activity of Rac-1 in the presence of the rs34932801 (G>C) SNP in the RAC1 promoter, and interestingly, this polymorphism was associated with MP hematologic toxicity in a cohort of European IBD patients[44]. Another study reported that Rac-1 expression levels decreased during thiopurine maintenance therapy in IBD patients and that MP responders presented lower Rac-1 expression and activity levels, whereas in nonresponders, these parameters were increased. On these bases, Rac-1 was proposed as a potential biomarker of thiopurine effectiveness in IBD[45]. Intriguingly, conditional disruption of Rac-1 in phagocytes of mice resulted in protection from colitis[46]. In contrast, Rac-1 and STAT3 signaling have been considered contributing factors to IBD development[47], and it was found that both the expression and activity levels of Rac-1 were directly related to colon inflammation grade[46]. Sustained Rac-1-GTP activity in lamina propria T lymphocytes could be more difficult to counteract by thiopurines and lead to resistance of T lymphocytes to apoptosis and thus to their unrestrained accumulation, which subsequently results in the amplification of the inflammatory response in the GI tract. In this sense, in IBD patients, Rac-1 could represent a biomarker of thiopurine-induced GI toxicity and of disease severity and progression, without a clear discrimination between the two clinical phenotypes.

Another potential biomarker for thiopurine GI toxicity is the inosine triphosphate pyrophosphatase (ITPA) gene. ITPA is one of the enzymes involved in the thiopurine metabolic pathway. By hydrolyzing inosine triphosphate (ITP) and xanthosine triphosphate nucleotides (XTP) into their monophosphate derivatives (IMP and XMP, respectively), ITPA prevents the accumulation of these noncanonical metabolites in cells and their incorporation into DNA or RNA, where they can interact with DNA/RNA polymerase activity[48]. The thioinosine analog (tIMP), an intermediate of MP conversion to TGN, is converted to tITP, which is also an ITPA substrate (Figure 1). A study performed on a large childhood ALL cohort (n = 511), treated according to the AIEOP-BFM-2000 protocol, showed that the missense variant rs1127354 (C>A, p. Pro32Thr) in ITPA was associated with a higher risk of severe GI toxicity during induction/consolidation therapy[10]. This missense variant partially reduces ITPA enzymatic activity in heterozygotes and completely reduces ITPA enzymatic activity in variant homozygotes[49,50], stimulating the accumulation of unusual tITP with the potential to cause adverse metabolic effects[51]. Other studies in pediatric ALL patients showed contradictory results on the ITPA rs1127354 association with myelotoxicity[52-54]. Stocco et al[53] found significantly higher concentrations of MMPN in patients with the nonfunctional ITPA allele. The association between the ITPA polymorphism and MP metabolism or neutropenia in ALL patients treated with an MP dose adjusted on the basis of the TPMT genotype underlined the important role of this gene in thiopurine toxicity.

**CLINICAL IMPLEMENTATIONS**

The PACSIN2 rs2413739 SNP could be considered a potential biomarker for thiopurine-related GI toxicity, being associated with this clinical phenotype in three independent ALL cohorts and with increased active disease in a cohort of IBD patients. Further investigations are needed to understand the molecular basis of this genetic effect and the functional role of the PACSIN2 protein in the healthy and damaged GI epithelium before its possible translation into the clinic. Additionally, the contribution of RAC1 and ITPA SNPs, as potential biomarkers for thiopurine-related GI toxicity, requires further validation in patients undergoing therapy with these drugs. Currently, there are no clinical trials focusing on the role of these genes/proteins in GI toxicity in ALL and IBD patients.

If these candidates would be confirmed as markers for GI toxicity, several applications could be speculated in clinical practice. For example, in patients treated with thiopurines, clinicians could be warned of the patients’ genetic predisposition to GI damage (e.g., patients carrying the PACSIN2 rs2413739 or ITPA rs1127354 homozygous variant genotypes). Pharmacogenetic information could be used as an alert for physicians, identifying patients who need intensive monitoring for adverse effects or those who should undergo supportive care earlier, even when less severe episodes of toxicity occur.
CONCLUSION

While highly effective, thiopurines are responsible for serious toxicities in ALL and IBD. This scenario points out the importance of identifying predictive biomarkers for detecting and monitoring the tissue-specific side effects of thiopurine. Data reported in this editorial underline the complexity of thiopurine pharmacokinetic mechanisms, which could be influenced by multiple genes and nongenetic factors able to exert their function on the whole body or through a tissue-specific mechanism of action.

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