World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2021 October 14; 27(38): 6348-6356

DOI: 10.3748/wjg.v27.i38.6348

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Biomarkers for gastrointestinal adverse events related to thiopurine therapy

Giulia Zudeh, Raffaella Franca, Gabriele Stocco, Giuliana Decorti

ORCID number: Giulia Zudeh 0000-0001-6168-5662; Raffaella Franca 0000-0001-8569-2023; Gabriele Stocco 0000-0003-0964-5879; Giuliana Decorti 0000-0002-9714-6246.

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.

Manuscript source: Invited

Giulia Zudeh, Gabriele Stocco, Department of Life Sciences, University of Trieste, Trieste 34127, Italy

Raffaella Franca, Giuliana Decorti, Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste 34149, Italy

Giuliana Decorti, Institute for Maternal and Child Health I.R.C.C.S Burlo Garofolo, Trieste 34137, Italy

Corresponding author: Gabriele Stocco, PhD, Associate Professor, Department of Life Sciences, University of Trieste, Via Fleming 22, Trieste 34127, Italy. stoccog@units.it

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 *PACSIN2*, *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; *PACSIN2*; *RAC1*; *ITPA*

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 *PACSIN2*, *RAC1*, and *ITPA* genes, in addition to *TPMT* and *NUDT15*, as possible

manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Italy

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: March 12, 2021
Peer-review started: March 12, 2021
First decision: April 17, 2021
Revised: April 29, 2021
Accepted: September 3, 2021
Article in press: September 3, 2021
Published online: October 14, 2021

P-Reviewer: Dahiya DS
S-Editor: Gao CC
L-Editor: Wang TQ
P-Editor: Yu HG

biomarkers for thiopurine-related gastrointestinal toxicity.

Citation: Zudeh G, Franca R, Stocco G, Decorti G. Biomarkers for gastrointestinal adverse events related to thiopurine therapy. *World J Gastroenterol* 2021; 27(38): 6348-6356
URL: https://www.wjgnet.com/1007-9327/full/v27/i38/6348.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i38.6348

INTRODUCTION

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 rush[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, pAla80Pro), *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

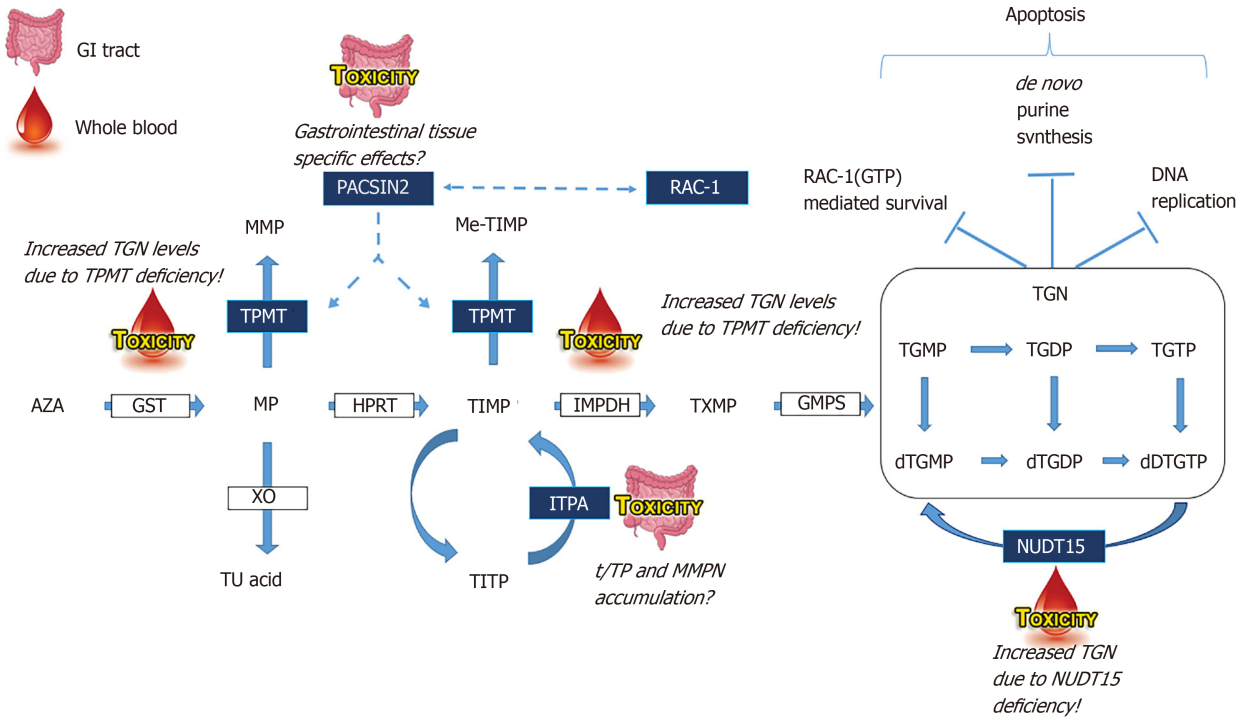

Figure 1 Thiopurine metabolic pathway and possible biomarkers for drug-related toxicity. Dashed arrows indicate the impact of PACSIN2 on TPMT activity and the interaction between PACSIN2 and Rac-1. AZA: Azathioprine; ITPA: Inosine triphosphate pyrophosphatase; IMPDH: Inosine-5'-monophosphate dehydrogenase; GMPS: GMP synthase; GST: Glutathione-S-transferase; Me-TIMP: Methyl-thioinosine monophosphate; Me-TITP: Methyl-thioinosinetriphosphate; MP: Mercaptopurine; MMP: Methyl-mercaptopurine; NUDT15: Nudix hydrolase 15; PACSIN2: Protein kinase C and casein kinase substrate in neurons protein 2; TGDP: Thioguanine diphosphate; TGMP: Thioguanine monophosphate; TGTP: Thioguanine triphosphate; TIMP: Thioinosine monophosphate; TITP: Thioinosine triphosphate; TNG: 6-Thioguanine nucleotide; TPMT: Thiopurine S-methyltransferase; TXMP: Thioxanthine monophosphate; 6-TU acid: Thiouric acid; XO: Xanthine oxidase.

variants are indeed associated with a higher risk of myelosuppression[21]. Variable number tandem repeats (VNTRs) in the *TPMT* promoter are associated with reduced *TPMT* expression levels and a higher risk of MP hematological toxicity[22]. Furthermore, genetic variants in nudix hydrolase 15 (*NUDT15*) have been identified as additional pharmacogenetic markers for the prediction of thiopurine-induced toxicities, especially in Asian individuals. NUDT15 removes a pyrophosphate group by canonical GTP and drug-derived tGTP active metabolites. The most studied *NUDT15* variants are rs116855232 (c.415C> T, p. Arg139Cys), rs147390019 (G>A, p. Arg139His), rs186364861 (G>A, p. Val18Ile), and rs746071566 (36_37insGGAGTC insertion, p.Val18_Val19insGlyVal). Variant alleles encode NUDT15 with compromised activity, leading to a higher tGTP/tGMP ratio and incorporation of TGN into DNA[23,24]. Indeed, these variants have been associated with MP and AZA intolerance[23,25]. On these bases, different guidelines for thiopurine dose adjustment based on *TPMT* and *NUDT15* genotypes have been released to reduce the occurrence of drug-related side effects[26].

In addition to pharmacogenetic markers, TGN levels could be monitored in erythrocytes to avoid severe myelosuppression during therapy. In particular, TGN levels higher than 450 pmol/8 × 10^8 red blood cells (RBCs) and higher than 1000 pmol/8 × 10^8 RBCs have been shown to be associated with myelotoxicity in IBD and ALL patients, respectively, while levels of MMPN above 5700 pmol/8 × 10^8 RBCs have been shown to be related to a higher hepatotoxicity risk in IBD patients[27,28].

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 (SJRCH, 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 SJRCH Total 13B protocol and to the Associazione Italiana Ematologia Oncologia Pediatrica/Berlin-Frankfurt-Münster (AIEOP-BFM) 2000 protocol[32]. Patients received 75 mg/m^2 MP daily and 2 g/m^2 high-dose methotrexate (HD-MTX) i.v. twice a week for 2 wk at SJRCH, whereas those undergoing the AIEOP-BFM 2000 protocol were treated daily with 25 mg/m^2 MP and received four HD-MTX (2-5 g/m^2) 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 can overlap 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.

REFERENCES

1 **Cooper SL**, Brown PA. Treatment of pediatric acute lymphoblastic leukemia. *Pediatr Clin North Am* 2015; **62**: 61-73 [PMID: 25435112 DOI: 10.1016/j.pcl.2014.09.006]
2 **Chande N**, Patton PH, Tsoulis DJ, Thomas BS, MacDonald JK. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2015; CD000067 [PMID: 26517527 DOI: 10.1002/14651858.CD000067.pub3]
3 **Tiede I**, Fritz G, Strand S, Poppe D, Dvorsky R, Strand D, Lehr HA, Wirtz S, Becker C, Atreya R, Mudter J, Hildner K, Bartsch B, Holtmann M, Blumberg R, Walczak H, Iven H, Galle PR, Ahmadian MR, Neurath MF. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. *J Clin Invest* 2003; **111**: 1133-1145 [PMID: 12697733 DOI: 10.1172/JCI16432]
4 **Coulthard S**, Hogarth L. The thiopurines: an update. *Invest New Drugs* 2005; **23**: 523-532 [PMID: 16267626 DOI: 10.1007/s10637-005-4020-8]
5 **Hindorf U**, Lindqvist M, Peterson C, Söderkvist P, Ström M, Hjortswang H, Pousette A, Almer S. Pharmacogenetics during standardised initiation of thiopurine treatment in inflammatory bowel disease. *Gut* 2006; **55**: 1423-1431 [PMID: 16543290 DOI: 10.1136/gut.2005.074930]
6 **An Q**, Fan CH, Xu SM. Current views of common pediatric cancers - an update. *Eur Rev Med Pharmacol Sci* 2017; **21**: 20-24 [PMID: 29165770]
7 **de Boer NKH**, Peyrin-Biroulet L, Jharap B, Sanderson JD, Meijer B, Atreya I, Barclay ML, Colombel JF, Lopez A, Beaugerie L, Marinaki AM, van Bodegraven AA, Neurath MF. Thiopurines in Inflammatory Bowel Disease: New Findings and Perspectives. *J Crohns Colitis* 2018; **12**: 610-620 [PMID: 29293971 DOI: 10.1093/ecco-jcc/jjx181]
8 **Lennard L**, Rees CA, Lilleyman JS, Maddocks JL. Childhood leukaemia: a relationship between intracellular 6-mercaptopurine metabolites and neutropenia. *Br J Clin Pharmacol* 1983; **16**: 359-363 [PMID: 6578834 DOI: 10.1111/j.1365-2125.1983.tb02178.x]
9 **Goel RM**, Blaker P, Mentzer A, Fong SC, Marinaki AM, Sanderson JD. Optimizing the use of thiopurines in inflammatory bowel disease. *Ther Adv Chronic Dis* 2015; **6**: 138-146 [PMID: 25954498 DOI: 10.1177/2040622315579063]
10 **Franca R**, Rebora P, Bertorello N, Fagioli F, Conter V, Biondi A, Colombini A, Micalizzi C, Zecca M, Parasole R, Petruzziello F, Basso G, Putti MC, Locatelli F, d'Adamo P, Valsecchi MG, Decorti G, Rabusin M. Pharmacogenetics and induction/consolidation therapy toxicities in acute lymphoblastic leukemia patients treated with AIEOP-BFM ALL 2000 protocol. *Pharmacogenomics J* 2017; **17**: 4-10 [PMID: 26644204 DOI: 10.1038/tpj.2015.83]
11 **Heckmann JM**, Lambson EM, Little F, Owen EP. Thiopurine methyltransferase (TPMT) heterozygosity and enzyme activity as predictive tests for the development of azathioprine-related adverse events. *J Neurol Sci* 2005; **231**: 71-80 [PMID: 15792824 DOI: 10.1016/j.jns.2005.01.003]
12 **Franca R**, Zudeh G, Lucafò M, Rabusin M, Decorti G, Stocco G. Genome wide association studies for treatment-related adverse effects of pediatric acute lymphoblastic leukemia. *Wiley Interdiscip Rev Syst Biol Med* 2020; e1509 [PMID: 33016644 DOI: 10.1002/wsbm.1509]
13 **Gisbert JP**, González-Lama Y, Maté J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. *Am J Gastroenterol* 2007; **102**: 1518-1527 [PMID: 17391318 DOI: 10.1111/j.1572-0241.2007.01187.x]
14 **Toksvang LN**, Schmidt MS, Arup S, Larsen RH, Frandsen TL, Schmiegelow K, Rank CU. Hepatotoxicity during 6-thioguanine treatment in inflammatory bowel disease and childhood acute lymphoblastic leukaemia: A systematic review. *PLoS One* 2019; **14**: e0212157 [PMID: 31125338 DOI: 10.1371/journal.pone.0212157]
15 **Chande N**, Townsend CM, Parker CE, MacDonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2016; **10**: CD000545 [PMID: 27783843 DOI: 10.1002/14651858.CD000545.pub5]
16 **Nygaard U**, Toft N, Schmiegelow K. Methylated metabolites of 6-mercaptopurine are associated with hepatotoxicity. *Clin Pharmacol Ther* 2004; **75**: 274-281 [PMID: 15060506 DOI: 10.1016/j.clpt.2003.12.001]
17 **Zhou S**. Clinical pharmacogenomics of thiopurine S-methyltransferase. *Curr Clin Pharmacol* 2006; **1**: 119-128 [PMID: 18666383 DOI: 10.2174/157488406784111627]
18 **Appell ML**, Berg J, Duley J, Evans WE, Kennedy MA, Lennard L, Marinaki T, McLeod HL, Relling MV, Schaeffeler E, Schwab M, Weinshilboum R, Yeoh AE, McDonagh EM, Hebert JM, Klein TE,

Coulthard SA. Nomenclature for alleles of the thiopurine methyltransferase gene. *Pharmacogenet Genomics* 2013; **23**: 242-248 [PMID: 23407052 DOI: 10.1097/FPC.0b013e32835f1cc0]

19 **Spire-Vayron de la Moureyre C**, Debuysere H, Mastain B, Vinner E, Marez D, Lo Guidice JM, Chevalier D, Brique S, Motte K, Colombel JF, Turck D, Noel C, Flipo RM, Pol A, Lhermitte M, Lafitte JJ, Libersa C, Broly F. Genotypic and phenotypic analysis of the polymorphic thiopurine S-methyltransferase gene (TPMT) in a European population. *Br J Pharmacol* 1998; **125**: 879-887 [PMID: 9831928 DOI: 10.1038/sj.bjp.0702152]

20 **Chouchana L**, Narjoz C, Roche D, Golmard JL, Pineau B, Chatellier G, Beaune P, Loriot MA. Interindividual variability in TPMT enzyme activity: 10 years of experience with thiopurine pharmacogenetics and therapeutic drug monitoring. *Pharmacogenomics* 2014; **15**: 745-757 [PMID: 24897283 DOI: 10.2217/pgs.14.32]

21 **Lennard L**, Cartwright CS, Wade R, Richards SM, Vora A. Thiopurine methyltransferase genotype-phenotype discordance and thiopurine active metabolite formation in childhood acute lymphoblastic leukaemia. *Br J Clin Pharmacol* 2013; **76**: 125-136 [PMID: 23252716 DOI: 10.1111/bcp.12066]

22 **Burgueño-Rodríguez G**, Méndez Y, Olano N, Dabezies A, Bertoni B, Souto J, Castillo L, da Luz J, Soler AM. Ancestry and TPMT-VNTR Polymorphism: Relationship with Hematological Toxicity in Uruguayan Patients with Acute Lymphoblastic Leukemia. *Front Pharmacol* 2020; **11**: 594262 [PMID: 33424606 DOI: 10.3389/fphar.2020.594262]

23 **Moriyama T**, Nishii R, Perez-Andreu V, Yang W, Klussmann FA, Zhao X, Lin TN, Hoshitsuki K, Nersting J, Kihira K, Hofmann U, Komada Y, Kato M, McCorkle R, Li L, Koh K, Najera CR, Kham SK, Isobe T, Chen Z, Chiew EK, Bhojwani D, Jeffries C, Lu Y, Schwab M, Inaba H, Pui CH, Relling MV, Manabe A, Hori H, Schmiegelow K, Yeoh AE, Evans WE, Yang JJ. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. *Nat Genet* 2016; **48**: 367-373 [PMID: 26878724 DOI: 10.1038/ng.3508]

24 **Suiter CC**, Moriyama T, Matreyek KA, Yang W, Scaletti ER, Nishii R, Hoshitsuki K, Singh M, Trehan A, Parish C, Smith C, Li L, Bhojwani D, Yuen LYP, Li CK, Li CH, Yang YL, Walker GJ, Goodhand JR, Kennedy NA, Klussmann FA, Bhatia S, Relling MV, Kato M, Hori H, Bhatia P, Ahmad T, Yeoh AEJ, Stenmark P, Fowler DM, Yang JJ. Massively parallel variant characterization identifies *NUDT15* alleles associated with thiopurine toxicity. *Proc Natl Acad Sci U S A* 2020; **117**: 5394-5401 [PMID: 32094176 DOI: 10.1073/pnas.1915680117]

25 **Moriyama T**, Nishii R, Lin TN, Kihira K, Toyoda H, Jacob N, Kato M, Koh K, Inaba H, Manabe A, Schmiegelow K, Yang JJ, Hori H. The effects of inherited NUDT15 polymorphisms on thiopurine active metabolites in Japanese children with acute lymphoblastic leukemia. *Pharmacogenet Genomics* 2017; **27**: 236-239 [PMID: 28445187 DOI: 10.1097/FPC.0000000000000282]

26 **Relling MV**, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui CH, Stein CM, Moyer AM, Evans WE, Klein TE, Antillon-Klussmann FG, Caudle KE, Kato M, Yeoh AEJ, Schmiegelow K, Yang JJ. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. *Clin Pharmacol Ther* 2019; **105**: 1095-1105 [PMID: 30447069 DOI: 10.1002/cpt.1304]

27 **Dubinsky MC**, Lamothe S, Yang HY, Targan SR, Sinnett D, Théorêt Y, Seidman EG. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology* 2000; **118**: 705-713 [PMID: 10734022 DOI: 10.1016/s0016-5085(00)70140-5]

28 **Adam de Beaumais T**, Fakhoury M, Medard Y, Azougagh S, Zhang D, Yakouben K, Jacqz-Aigrain E. Determinants of mercaptopurine toxicity in paediatric acute lymphoblastic leukemia maintenance therapy. *Br J Clin Pharmacol* 2011; **71**: 575-584 [PMID: 21395650 DOI: 10.1111/j.1365-2125.2010.03867.x]

29 **Liu C**, Yang W, Pei D, Cheng C, Smith C, Landier W, Hageman L, Chen Y, Yang JJ, Crews KR, Kornegay N, Karol SE, Wong FL, Jeha S, Sandlund JT, Ribeiro RC, Rubnitz JE, Metzger ML, Pui CH, Evans WE, Bhatia S, Relling MV. Genomewide Approach Validates Thiopurine Methyltransferase Activity Is a Monogenic Pharmacogenomic Trait. *Clin Pharmacol Ther* 2017; **101**: 373-381 [PMID: 27564568 DOI: 10.1002/cpt.463]

30 **McLeod HL**, Krynetski EY, Relling MV, Evans WE. Genetic polymorphism of thiopurine methyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia. *Leukemia* 2000; **14**: 567-572 [PMID: 10764140 DOI: 10.1038/sj.leu.2401723]

31 **Fakhoury M**, Andreu-Gallien J, Mahr A, Medard Y, Azougagh S, Vilmer E, Jacqz-Aigrain E. Should TPMT genotype and activity be used to monitor 6-mercaptopurine treatment in children with acute lymphoblastic leukaemia? *J Clin Pharm Ther* 2007; **32**: 633-639 [PMID: 18021342 DOI: 10.1111/j.1365-2710.2007.00858.x]

32 **Stocco G**, Yang W, Crews KR, Thierfelder WE, Decorti G, Londero M, Franca R, Rabusin M, Valsecchi MG, Pei D, Cheng C, Paugh SW, Ramsey LB, Diouf B, McCorkle JR, Jones TS, Pui CH, Relling MV, Evans WE. PACSIN2 polymorphism influences TPMT activity and mercaptopurine-related gastrointestinal toxicity. *Hum Mol Genet* 2012; **21**: 4793-4804 [PMID: 22846425 DOI: 10.1093/hmg/dds302]

33 **Franca R**, Stocco G, Favretto D, Giurici N, Del Rizzo I, Locatelli F, Vinti L, Biondi A, Colombini A, Fagioli F, Barisone E, Pelin M, Martellossi S, Ventura A, Decorti G, Rabusin M. PACSIN2 rs2413739 influence on thiopurine pharmacokinetics: validation studies in pediatric patients. *Pharmacogenomics J* 2020; **20**: 415-425 [PMID: 31792371 DOI: 10.1038/s41397-019-0130-0]

34 **McLeod HL**, Krynetski EY, Wilimas JA, Evans WE. Higher activity of polymorphic thiopurine S-

methyltransferase in erythrocytes from neonates compared to adults. *Pharmacogenetics* 1995; **5**: 281-286 [PMID: 8563768 DOI: 10.1097/00008571-199510000-00003]

35 **Pettersson B**, Almer S, Albertioni F, Söderhäll S, Peterson C. Differences between children and adults in thiopurine methyltransferase activity and metabolite formation during thiopurine therapy: possible role of concomitant methotrexate. *Ther Drug Monit* 2002; **24**: 351-358 [PMID: 12021625 DOI: 10.1097/00007691-200206000-00005]

36 **Karas-Kuželički N**, Šmid A, Tamm R, Metspalu A, Mlinarič-Raščan I. From pharmacogenetics to pharmacometabolomics: SAM modulates TPMT activity. *Pharmacogenomics* 2014; **15**: 1437-1449 [PMID: 25303295 DOI: 10.2217/pgs.14.84]

37 **GTEx Portal**. Genotype-Tissue Expression (GTEx) Portal. [cited 30 August 2021]. In: GTEx Portal [Internet]. Available from: https://gtexportal.org/home/

38 **Langer V**, Vivi E, Regensburger D, Winkler TH, Waldner MJ, Rath T, Schmid B, Skottke L, Lee S, Jeon NL, Wohlfahrt T, Kramer V, Tripal P, Schumann M, Kersting S, Handtrack C, Geppert CI, Suchowski K, Adams RH, Becker C, Ramming A, Naschberger E, Britzen-Laurent N, Stürzl M. IFN-γ drives inflammatory bowel disease pathogenesis through VE-cadherin-directed vascular barrier disruption. *J Clin Invest* 2019; **129**: 4691-4707 [PMID: 31566580 DOI: 10.1172/JCI124884]

39 **Dorland YL**, Malinova TS, van Stalborch AM, Grieve AG, van Geemen D, Jansen NS, de Kreuk BJ, Nawaz K, Kole J, Geerts D, Musters RJ, de Rooij J, Hordijk PL, Huveneers S. The F-BAR protein pacsin2 inhibits asymmetric VE-cadherin internalization from tensile adherens junctions. *Nat Commun* 2016; **7**: 12210 [PMID: 27417273 DOI: 10.1038/ncomms12210]

40 **Iida T**, Onodera K, Nakase H. Role of autophagy in the pathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2017; **23**: 1944-1953 [PMID: 28373760 DOI: 10.3748/wjg.v23.i11.1944]

41 **Haq S**, Grondin J, Banskota S, Khan WI. Autophagy: roles in intestinal mucosal homeostasis and inflammation. *J Biomed Sci* 2019; **26**: 19 [PMID: 30764829 DOI: 10.1186/s12929-019-0512-2]

42 **Uhlén M**, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Pontén F. Proteomics. Tissue-based map of the human proteome. *Science* 2015; **347**: 1260419 [PMID: 25613900 DOI: 10.1126/science.1260419]

43 **de Kreuk BJ**, Nethe M, Fernandez-Borja M, Anthony EC, Hensbergen PJ, Deelder AM, Plomann M, Hordijk PL. The F-BAR domain protein PACSIN2 associates with Rac1 and regulates cell spreading and migration. *J Cell Sci* 2011; **124**: 2375-2388 [PMID: 21693584 DOI: 10.1242/jcs.080630]

44 **Bourgine J**, Garat A, Allorge D, Crunelle-Thibaut A, Lo-Guidice JM, Colombel JF, Broly F, Billaut-Laden I. Evidence for a functional genetic polymorphism of the Rho-GTPase Rac1. Implication in azathioprine response? *Pharmacogenet Genomics* 2011; **21**: 313-324 [PMID: 21372752 DOI: 10.1097/FPC.0b013e3283449200]

45 **Seinen ML**, van Nieuw Amerongen GP, de Boer NK, Mulder CJ, van Bezu J, van Bodegraven AA. Rac1 as a Potential Pharmacodynamic Biomarker for Thiopurine Therapy in Inflammatory Bowel Disease. *Ther Drug Monit* 2016; **38**: 621-627 [PMID: 27465973 DOI: 10.1097/FTD.0000000000000326]

46 **Muise AM**, Walters T, Xu W, Shen-Tu G, Guo CH, Fattouh R, Lam GY, Wolters VM, Bennitz J, van Limbergen J, Renbaum P, Kasirer Y, Ngan BY, Turner D, Denson LA, Sherman PM, Duerr RH, Cho J, Lees CW, Satsangi J, Wilson DC, Paterson AD, Griffiths AM, Glogauer M, Silverberg MS, Brumell JH. Single nucleotide polymorphisms that increase expression of the guanosine triphosphatase RAC1 are associated with ulcerative colitis. *Gastroenterology* 2011; **141**: 633-641 [PMID: 21684284 DOI: 10.1053/j.gastro.2011.04.057]

47 **Atreya R**, Atreya I, Neurath MF. Novel signal transduction pathways: analysis of STAT-3 and Rac-1 signaling in inflammatory bowel disease. *Ann N Y Acad Sci* 2006; **1072**: 98-113 [PMID: 17057193 DOI: 10.1196/annals.1326.001]

48 **Sakumi K**, Abolhassani N, Behmanesh M, Iyama T, Tsuchimoto D, Nakabeppu Y. ITPA protein, an enzyme that eliminates deaminated purine nucleoside triphosphates in cells. *Mutat Res* 2010; **703**: 43-50 [PMID: 20601097 DOI: 10.1016/j.mrgentox.2010.06.009]

49 **Shipkova M**, Lorenz K, Oellerich M, Wieland E, von Ahsen N. Measurement of erythrocyte inosine triphosphate pyrophosphohydrolase (ITPA) activity by HPLC and correlation of ITPA genotype-phenotype in a Caucasian population. *Clin Chem* 2006; **52**: 240-247 [PMID: 16384889 DOI: 10.1373/clinchem.2005.059501]

50 **Arenas M**, Duley J, Sumi S, Sanderson J, Marinaki A. The ITPA c.94C>A and g.IVS2+21A>C sequence variants contribute to missplicing of the ITPA gene. *Biochim Biophys Acta* 2007; **1772**: 96-102 [PMID: 17113761 DOI: 10.1016/j.bbadis.2006.10.006]

51 **Marinaki AM**, Ansari A, Duley JA, Arenas M, Sumi S, Lewis CM, Shobowale-Bakre el-M, Escuredo E, Fairbanks LD, Sanderson JD. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). *Pharmacogenetics* 2004; **14**: 181-187 [PMID: 15167706 DOI: 10.1097/00008571-200403000-00006]

52 **Hareedy MS**, El Desoky ES, Woillard JB, Thabet RH, Ali AM, Marquet P, Picard N. Genetic variants in 6-mercaptopurine pathway as potential factors of hematological toxicity in acute lymphoblastic leukemia patients. *Pharmacogenomics* 2015; **16**: 1119-1134 [PMID: 26237184 DOI: 10.2217/PGS.15.62]

53 **Stocco G**, Cheok MH, Crews KR, Dervieux T, French D, Pei D, Yang W, Cheng C, Pui CH, Relling

MV, Evans WE. Genetic polymorphism of inosine triphosphate pyrophosphatase is a determinant of mercaptopurine metabolism and toxicity during treatment for acute lymphoblastic leukemia. *Clin Pharmacol Ther* 2009; **85**: 164-172 [PMID: 18685564 DOI: 10.1038/clpt.2008.154]

54 **Chiengthong K**, Ittiwut C, Muensri S, Sophonphan J, Sosothikul D, Seksan P, Suppipat K, Suphapeetiporn K, Shotelersuk V. NUDT15 c.415C>T increases risk of 6-mercaptopurine induced myelosuppression during maintenance therapy in children with acute lymphoblastic leukemia. *Haematologica* 2016; **101**: e24-e26 [PMID: 26405151 DOI: 10.3324/haematol.2015.134775]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com