Review

Personalization of thiopurine therapy: Current recommendations and future perspectives

DUNJA URBANČIČ[1]
FLAKA PASHA[1,2]
ALENKA ŠMID[1]
IRENA MLINARIČ-RAŠČAN[1,*]

[1] *University of Ljubljana, Faculty of Pharmacy, 1000 Ljubljana, Slovenia*

[2] *University of Prishtina "Hasan Prishtina", Faculty of Medicine Department of Pharmacology with Toxicology, and Clinical Pharmacology 10000 Prishtina, Kosovo*

ABSTRACT

Despite great therapeutic advances in the field of biologics, small synthetic molecules such as thiopurines, including azathioprine, mercaptopurine, and thioguanine, remain an important therapeutic pillar in the treatment of inflammatory bowel disease, other autoimmune disorders, and cancer. This review presents the latest guidelines for thiopurine administration, highlighting the importance of individualized therapy guided by pharmacogenomics. It emphasizes dose adjustment based on nudix hydrolase 15 (*NUDT15*) and thiopurine *S*-methyltransferase (*TPMT*) genotype, alongside thiopurine *S*-methyltransferase activity and thiopurine metabolic profile. In addition, the article takes a critical look at emerging research in the field of thiopurine pharmacogenomics featuring novel genetic markers and technological developments in genetic testing. Finally, the potential of integrated approaches that combine genetic, metabolic, and clinical factors to further individualize thiopurine therapy is highlighted.

Keywords: thiopurines, TPMT, NUDT15, personalized medicine, therapeutic drug monitoring

INTRODUCTION

Thiopurines were first discovered by Gertrude B. Elion and George H. Hitchings in the early 1950s (1). For their groundbreaking contributions to drug development, the pair was awarded the Nobel Prize in Medicine or Physiology in 1988. Since their discovery, thiopurines, including azathioprine (AZA), 6-mercaptopurine (6-MP), and 6-thioguanine (6-TG), have demonstrated their efficacy in various clinical applications. Thiopurines continue to be widely used in the treatment of chronic systemic autoimmune diseases, and prevention of transplant rejection, and remain an integral part of treatment for acute lymphoblastic leukemia (ALL) (2, 3).

Thiopurines are used to maintain remission, especially of corticosteroid-dependent or refractory inflammatory bowel disease (IBD) in adult patients (2). While the introduction

* Correspondence; e-mail address: irena.mlinaric@ffa.uni-lj.si

of biologics has changed the therapeutic landscape of IBD, thiopurines continue to be an essential, efficient, and cost-effective component of IBD treatment, either as monotherapy or in combination with other treatments, particularly infliximab (2, 4–7). In the treatment protocol for childhood acute lymphoblastic leukemia (ALL), 6-MP serves as the backbone during the final and most prolonged phase of AIEOP BFM ALL 2017 protocol, the maintenance phase, which lasts up to two years (3). The continued relevance of thiopurines in clinical practice is further supported by their use in the treatment of other diseases such as acute myeloid leukemia, various lymphomas, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, eczema, autoimmune hepatitis, idiopathic thrombocytopenia, and transplant medicine.

Thiopurines therefore remain an important anticancer and immunosuppressive group of drugs. However, they are characterized by a complex metabolism and narrow therapeutic index that exposes patients to the risk of severe, potentially life-threatening side-effects (8, 9). Due to individual genetic or metabolic differences, the same dose may achieve the desired effect in one patient while provoking a serious adverse reaction in another (10, 11). Therefore, precise dosing is an important factor to consider when using them. To address this challenge, pharmacogenomic guidelines for thiopurine *S*-methyltransferase (*TPMT*) genetic polymorphisms and TPMT activity have been in place for more than a decade (12), while recent advances in genomics also include the determination of the most common genetic polymorphisms in nudix hydrolase 15 (*NUDT15*) (11). In addition, therapeutic drug monitoring that allows quantification of thiopurine metabolites, especially 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MMP), is an important component in the optimization of thiopurine treatment if there is no response to thiopurines and/or in case of side-effects especially during IBD treatment (2, 13).

In this review, we highlight biomarkers for thiopurine therapy, namely, genetic polymorphisms in *TPMT* and *NUDT15*, and thiopurine metabolites. We first focus on the molecular basis of genotype-phenotype correlations and how these associations have led to well-established guidelines for thiopurine dosing. We then describe methods and clinically validated analytical procedures for *TPMT* and *NUDT15* genotyping, measurement of TPMT activity, and monitoring of thiopurine metabolites. Finally, we address future challenges, particularly in relation to clinical implementation, and what are the approaches to overcome them.

THIOPURINES

Treatment with thiopurines lasts from one to several years, primarily during the maintenance phases of ALL and IBD, with the aim of extending disease remission. The most common starting daily dose of 6-MP in the maintenance phase of ALL treatment is 50 to 75 mg m^{-2} (1.5 to 2.5 mg kg^{-1}), while IBD patients usually receive 1–3 mg kg^{-1} of AZA for maintaining remission (4, 14). The effectiveness of thiopurine treatment varies and depends on the type of disease and the age of patients. Due to a systematic approach to designing treatment protocols, the effectiveness in the field of ALL has increased from 40 % in the mid-1960s (15) to over 90 % in the pediatric population in recent years (3, 16). Nevertheless, some studies report only a 35 % effectiveness rate for thiopurine treatment in IBD (17, 18). Up to 60 % of patients discontinue treatment due to severe adverse effects, such as hypersensitivity reactions, acute pancreatitis, gastrointestinal toxicity, leukopenia, hepatotoxicity, and infections, or non-response to thiopurines (19, 20).

Metabolic pathways

Thiopurines are antitumor and immunosuppressive prodrugs, that undergo complex metabolism involving both activation and deactivation pathways (Fig. 1). After oral administration, the conversion of thiopurines begins in the intestines and liver, where AZA is partially enzymatically cleaved by glutatione *S*-transferase (GST) forming methyl nitrothio-imidazole and 6-MP. The latter may enter the activation pathway resulting in the production of cytotoxic 6-TGN. This process starts with the attachment of ribosyl and phosphate groups to the purine moiety by hypoxanthine phosphoribosyltransferase (HPRT) and continues with an oxidative reaction catalyzed by inosine 5'-monophosphate dehydrogenase (IMPDH), which significantly influences the conversion rate to 6-TGN. The next step involves the conversion of the resulting 6-thioinosine monophosphate (TIMP) to 6-thioguanosine monophosphate (TGMP) by guanosine 5'-monophosphate synthase (GMPS). This is followed by phosphorylation by kinases to 6-thioguanosine triphosphate (TGTP) (9). This latter step is opposed by dephosphorylation with the NUDT15 (21).

Apart from the activation, there are two deactivation pathways of thiopurines, involving TPMT and xanthine oxidase (XO) (9). As a liver enzyme, XO converts between 3 and 37 % of absorbed 6-MP into thiouric acid (10). The remainder of 6-MP then either enters the activation
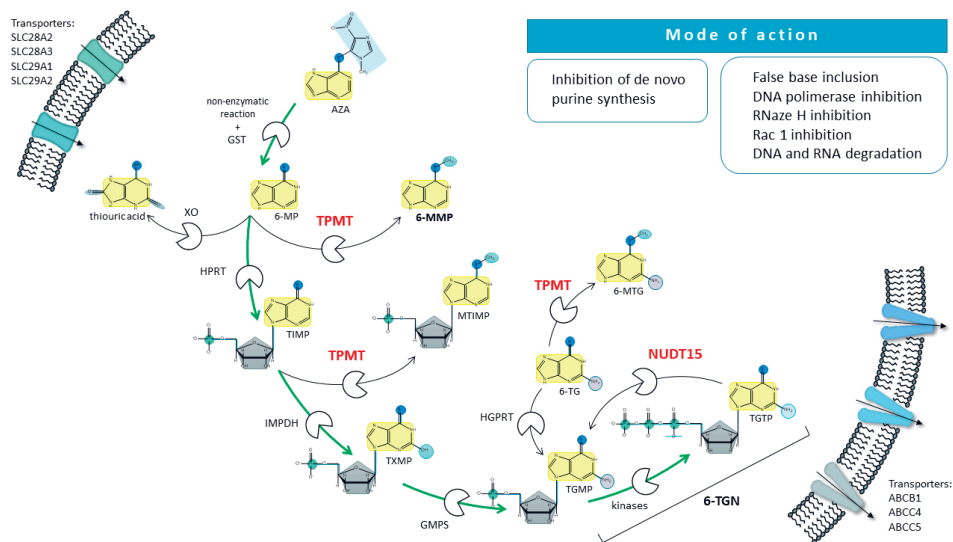

Fig. 1. Schematic presentation of thiopurine metabolism. 6-MP – 6-mercaptopurine, 6-MMP – 6-methyl-mercaptopurine, 6-MTG – 6-methylthioguanine, 6-TG – 6-tioguanine, 6-TGN – 6-tioguanine nucleotides, ABC – ATP-binding cassette (transporters), AZA – azathioprine, GMPS – guanosine monophosphate synthetase, GST – glutatione *S*-transferase, HPRT – hypoxanthine-guanine phosphoribosyltransferase, IMPDH – inosine-5'-monophosphate dehydrogenase, ITPA – inosine triphosphate pyrophosphatase, MTIMP – methylthioinosine monophosphate, NUDT15 – nudix hydrolase 15, XO – xanthine oxidase, SLC – solute carrier (transporters), TIMP – thioinosine monophosphate, TGMP – thioguanosine monophosphate, TGTP – thioguanosine triphosphate, TPMT – thiopurine *S*-methyltransferase, TXMP – thioxanthine monophosphate.

pathway or is methylated by TPMT, resulting in the formation of 6-MMP. TPMT also partici-pates in the methylation of some other 6-MP metabolites at various stages of the activation pathway (Fig. 1) (9). Namely, it is involved in the production of 6-methylthioinosine mono-phosphate (MTIMP), which, alongside 6-TGN, exhibits pharmacological effects (22).

The activation and deactivation pathways for 6-TG are similar to the metabolism of 6-MP but are less complex since 6-TG, unlike 6-MP, already holds a guanine structure. Only a one-step conversion by HPRT transforms 6-TG into cytotoxic 6-TGN (Fig. 1) (9). The oxidative deactivation of 6-TG by XO has not been fully elucidated. 6-TG is also deactivated by TPMT methylation; however, its methylated metabolites do not demonstrate pharma-cological activity (23).

Mode of action

Due to its structural similarity to guanine, 6-TGN incorporates as a building block into nucleic acids; however, instead of participating in synthesis, it disrupts the function of DNA polymerase, and thereby arrests replication of DNA, causing DNA degradation (24). The activity of 6-TGN includes the direct inhibition of DNA polymerase and ligase, as well as the suppression of RNase H activity, leading to a termination in gene transcription (25). The disintegration of nucleic acids triggers apoptotic mechanisms and cell death. The cytostatic and immunosuppressive effects of thiopurines, specifically TGTP, are also achieved through the obstruction of CD28-dependent activation of Rac1, which directs T lymphocytes toward apoptosis (26). Additional activity of thiopurines is expressed by MTIMP. By inhibiting phosphoribosyl pyrophosphate amidotransferase, it suppresses *de novo* purine synthesis and thereby reduces the level of adenosine triphosphate (22). Another target effect of thiopurines is triggered by methyl nitrothioimidazole, a degrada-tion product of AZA. Its immunosuppressive effect is associated with the inhibition of oxidative stress defense mechanisms, as it can reduce the concentration of glutathione in the cell (27).

Safety and efficacy of treatment with thiopurines

Among patients treated with thiopurines, the most common motives for discontinu-ing therapy include hypersensitivity reactions, acute pancreatitis, gastrointestinal intole-rance, myelotoxicity manifested as neutropenia, hepatotoxicity, and infections (28). Due to the potential mutagenic and carcinogenic activity of thiopurines, some patients who have successfully undergone treatment later reported developing secondary neoplasms, such as lymphoma, non-melanoma skin tumors, and chronic myeloid leukemia (29–31).

To ensure patient safety and efficacy of treatment with thiopurines, routine checks of the leukocyte levels and liver function through liver enzymes are mandatory during treat-ment, usually every three months in patients with IBD (32) and every 8–12 weeks during maintenance treatment of ALL (33). Where available, the level of thiopurine metabolites in hemolysates is frequently monitored in patients unresponsive to treatment or in indivi-duals exhibiting side-effects (2, 13, 34–36). Levels of 6-TGN in the range of 235 to 450 pmol/8×10^8 red blood cells (RBC) in patients with IBD predict successful treatment (37). 6-TGN levels lower than 235 pmol/8×10^8 RBC are associated with non-responsiveness to therapy or indicate non-compliance (38, 39). Levels of 6-TGN higher than 450 pmol/8×10^8

RBC are associated with an increased risk of adverse effects (40, 41). Significantly elevated methylated thiopurine metabolites – 6-MMP > 5700 pmol/8×10^8 RBC, indicate a higher risk for liver toxicity, although monitoring 6-MMP alone is insufficient for predicting hepato-toxicity (4, 37, 42). A high ratio of 6-MMP to 6-TGN has proven useful, especially for detecting non-responders or shunters, a subgroup of patients, that excessively produce 6-MMP instead of 6-TGN.

PHARMACOGENOMIC DETERMINANTS OF THIOPURINE METABOLISM

Alongside the nature of the disease, inherited predisposition significantly influences the acute onset of adverse effects, disease relapse, adverse effects that occur years after completion of therapy, and non-response to thiopurines. Genetic polymorphisms are one of the factors that can lead to insufficient efficacy and decreased safety of the drug when different individuals are exposed to the same dose (45). Clinical and translational studies have identified a number of polymorphisms affecting the clinical response to thiopurines, correlating with their efficacy, toxicity, or resistance to such treatment (8). However, only variants in *TPMT* and *NUDT15* have been translated into clinical practice (11). We highlight these two genes and their implication in thiopurine therapy in the following sections.

Thiopurine S-methyltransferase

Thiopurine *S*-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes the *S*-methylation of aromatic and heteroaromatic sulfhydryl compounds, with the methyl group being donated by the co-factor *S*-adenosyl methionine (SAM) (46, 47). The reaction, which presumably follows the classic nucleophilic substitution (SN2) mechanism (48), results in the formation of a methylated substrate and *S*-adenosyl homocysteine (SAH) (46). SAM serves not only as a methyl donor but also stabilizes TPMT, preserving its enzymatic activity, which was highlighted in our previous studies (49, 50). The gene encoding this protein is located on chromosome 6 at locus 6p22.3. The gene spans over 34 kb and consists of 9 exons, of which 8 code for the protein (51). In the human genome, pseudogenes for *TPMT* are located on chromosomes 18 and X (51).

Currently, 46 different single nucleotide polymorphisms (SNPs) as well as deletions of smaller gene fragments are known in the exonic or intronic region (52, 53). In addition to the SNPs, individuals also differ in the sequence of the *TPMT* promoter region with a G/C--rich variable number of tandem repeats (VNTRs), which are evolutionarily related to the most common SNPs (54). Due to their impact on enzyme activity and their significant frequency, the most important genetic polymorphisms are *TPMT*3A*, *TPMT*3C*, and *TPMT*2* (55–58). In these polymorphisms, a change in one nucleotide leads to a protein with an altered amino acid sequence, resulting in incorrect folding and faster degradation of TPMT (59). Consequently, the activity of TPMT corresponds to the individual's genotype, where individuals with wild-type *TPMT* have normal TPMT activity, heterozygous individuals have intermediate TPMT activity, and individuals carrying both variant alleles express TPMT with barely detectable enzymatic activity (58). In the European population, about 89 % of individuals have normal TPMT activity, 11 % have reduced TPMT activity, and around 0.3 % have very low TPMT activity (10, 58). However, the distribution of activities in individuals with the wild-type genotype is relatively wide, meaning that the

genotype of the most common polymorphisms does not perfectly explain the phenotype and that there are other genetic or non-genetic factors that additionally influence TPMT activity (60).

Nudix hydrolase 15

Nudix hydrolase 15 (NUDT15) is an enzyme that belongs to the nudix hydrolase family of enzymes encoded by 24 genes on the human genome, which contains also at least 5 pseudogenes (61). These enzymes catalyze the hydrolysis of substrates with a general nucleoside diphosphate structure linked to another moiety, such as canonical and oxidized derivatives of (d)NTPs, nucleotide sugars, and dinucleotide polyphosphates. They share an evolutionary conserved domain named NUDIX box consisting of 23 amino acids, among which glutamic acid residues bind divalent cations, while other amino acids contribute to substrate specificity (61). *In vitro* studies have shown that NUDT15 preferentially hydrolyses 6-thio-deoxyguanosine triphosphate (6-TdGTP) and TGTP, the active metabolites of thiopurines, and negatively affects their desired cytotoxic and immunosuppressive effects, as well as increases the risk of thiopurine-related toxicities (62). Spanning over 15,030 base pairs on chromosome 13, the *NUDT15* gene has 3 exons and corresponding intronic regions (63). The first association of *NUDT15* variant (rs116855232) with thiopurine-induced toxicity came from a GWAS conducted in Korean patients with IBD (64) and was later confirmed in several other studies in patients with IBD and ALL (21, 65, 66). The identified missense variant c.415C>T, which results in a p.Arg139Cys change which leads to decreased enzyme activity, is present in *NUDT15*2* and *NUDT15*3* alleles. To date, several other variants have been identified and 20 different *NUDT15* alleles together with additional 7 suballeles have been reported in the Pharmacogene variant consortium database (67). Based on *in vitro* activity data or *in silico* prediction, alleles are divided into those having a normal function (*NUDT15*1* and it's 7 suballeles), alleles with severely decreased enzyme activity (*NUDT15*2 - *9*), alleles with no function (*NUDT15*10, *13, *14, *17, *18* and *19*) and alleles with unknown function (*NUDT15*11, *12, *15, *16* and *20)* (68).

Clinical implications and guidelines in thiopurine pharmacogenomics

Three *TPMT* alleles (*TPMT*2*, *TPMT*3A,* and *TPMT*3C*) constitute 90 % of cases of reduced activity phenotypes in individuals of European origin (10, 58, 69). As the presence of these alleles affects TPMT activity, poor TPMT metabolizers convert more thiopurines to the active 6-TGN, thus increasing the risk for drug toxicity, such as bone marrow suppression, severe gastric intolerance, and susceptibility to infections (70, 71). Similarly, individuals with genetic polymorphisms *NUDT15*2, NUDT15*3,* and *NUDT15*9* exhibit lower NUDT15 activity and a higher risk for drug toxicity when treated with thiopurines (21, 69). Therefore, thiopurines require tailored dosing strategies based on *TPMT* and *NUDT15* genetic variability. Genotyping of *TPMT* and *NUDT15* prior to treatment can mitigate adverse thiopurine effects such as myelosuppression and leukopenia, without compromising the disease's relapse rate (72–74).

International expert panels from the field of pharmacogenomics – Clinical Pharmacogenetics Implementation Consortium (CPIC), Dutch Pharmacogenetics Working Group (DPWG), and Canadian Pharmacogenomics Network for Drug Safety (CPNDS), have been established to consolidate information on how genetic polymorphisms impact treatment (11,

75, 76). These organizations have developed recommendations and pharmacogenomic guidelines for dosing of currently slightly less than 200 drugs, including thiopurines (75). Based on the functional impact of the variant alleles, minor allele frequencies in multiethnic populations, the availability of reference materials, and the feasibility for laboratories to test using standard methods, genetic polymorphisms in *TPMT* and *NUDT15* can be classified into two Tiers (77). Tier 1 variant alleles include *TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C*, and *NUDT15*3*, as they strongly affect enzyme activity, are tested by standard methods, and account for > 90 % or >50 % of the reported variant alleles, in *TPMT* or *NUDT15*, resp. Tier 2 includes other variant alleles in *TPMT* and *NUDT15* that are less frequent with less defined molecular mechanisms or difficulty to test for them by standard methods (77).

According to the presence of Tier 1 genetic polymorphisms in *TPMT* in *NUDT15*, guidelines classify individuals as normal metabolizers with two functional (non-variant) alleles, intermediate metabolizers with one functional and one non-functional (variant) allele, and poor metabolizers with two non-functional (variant) alleles in respective genes. CPIC guidelines further classify individuals with one allele of uncertain function and one non-functional allele (*i.e.*, *TPMT*2/TPMT*8, TPMT*3A/TPMT*7, NUDT15*2/NUDT15*5*, and *NUDT*3/NUDT15*6*) as possible intermediate metabolizers. According to guidelines (Table I), normal metabolizers should start with standard thiopurine doses, while intermediate metabolizers and possible intermediate metabolizers for either of the enzymes, should receive 30–80 % or 50–80 % of standard AZA/6-MP or 6-TG dose, resp. Poor metabolizers, diagnosed with malignancies, should start 6-MP at doses reduced by 10-fold thrice weekly, whereas in non-malignant cases it is suggested to consider alternative treatments to AZA. Alternative treatment is recommended also for poor metabolizers receiving 6-TG. In patients who are poor metabolizers due to the double variant *NUDT15*3* genotype, 25 % of the 6-TG normal dose is recommended for malignancies. Additional guidelines refer to patients who are intermediate metabolizers for both enzymes (TPMT and NUDT15) (11, 78–80). Such patients are suggested to start with a 20–50 % reduction of the thiopurine standard dose at the beginning of the treatment, followed by potential further dose adjustments based on the severity of myelosuppression (81).

Guidelines issued by various working groups exhibit minor discrepancies. For instance, according to the DPWG guidelines, dose reduction is not required for intermediate metabolizers of 6-MP or AZA when they are used to achieve immunosuppression, provided the initial dose does not exceed 1.5 mg kg^{-1} for AZA or 0.75 mg kg^{-1} per day for 6-MP. Additionally, the DPWG guidelines propose different dosing recommendations for 6-TG, according to the presence of genetic polymorphisms in *TPMT* or *NUDT15*. Dosing recommendations for thiopurines are summarized in Table I (75).

One of the risks associated with *TPMT* and *NUDT15* genotyping before prescribing thiopurines is the possibility of underdosing, as not all intermediate metabolizers experience myelosuppression at standard doses of thiopurines (82). Therefore, the guidelines also recommend monitoring the efficacy of thiopurine treatment and further adjusting the dose once the steady state is reached and according to routinely monitored clinical parameters such as leukocyte count (11, 75, 77).

Besides *TPMT* genotype tests, certain clinical laboratories offer also TPMT activity determination. Measuring TPMT activity has the advantage of identifying patients with high TPMT activity, and those who have low TPMT activity on account of other non-Tier

Table I. CPIC and DPWG recommendations on thiopurine dosing based on TPMT and NUDT15 metabolizer type, for Tier 1 variant alleles (11, 75, 77)

Allele	Metabolizer type (CPIC recommendations)				Metabolizer type (DPWG recommendations)	
	Normal	Intermediate for either TPMT or NUDT15	Intermediate for both enzymes	Poor	Intermediate	Poor
*TPMT*2* *TPMT*3A* *TPMT*3C*	Standard thiopurine dose	30–80 % of AZA/6-MP or 50–80 % of 6TG standard doses	20–50 % of thiopurine standard dose	Reduce daily dose by 10-fold, and dose thrice weekly for malignancies; consider alternative therapies for non-malignant cases	Start AZA at 50 %; no adjustments for doses below 1.5 mg kg^{-1} per day	Opt for an alternative treatment, or start AZA at 10 % of the standard dose
*NUDT15*3*				Reduce 6-MP/AZA by 10-fold; 25 % of the 6-TG normal dose for malignancies	Begin 6-TG at 75 %, adjust based on monitoring	Avoid 6-TG or start at 10 %

6-MP – 6-mercaptopurine, 6-TG – 6-thioguanine, AZA – azathioprine, CPIC – Clinical pharmacogenetics implementation consortium, DPWG – Dutch Pharmacogenetics Working Group, NUDT15 – nudix hydrolase 15, TPMT – thiopurine *S*-methyltransferase

1 genetic polymorphisms. Assays for testing NUDT15 enzyme activity are usually not integrated into clinical practice (77, 83).

Countries like the US, Canada, the UK, The Netherlands, France, Belgium, Spain, Australia, and New Zealand, highly recommend *TPMT* and *NUDT15* genotyping prior to treatment, due to their significant association with treatment outcomes (84–86). Additionally, St. Jude Children's Research Hospital is currently conducting a clinical trial entitled "*Total Therapy XVII for Newly Diagnosed Patients with Acute Lymphoblastic Leukemia and Lymphoma*", employing precision medicine strategies based on genomic features to improve cure rates and quality of life for children with ALL, highlighting the forever evolving importance of precision medicine (87).

ANALYTICAL METHODS FOR INDIVIDU-ALIZATION OF THIOPURINE THERAPY

Determination of TPMT and NUDT15 genotypes

Research studies that reported *TPMT* and *NUDT15* genotyping, employ mainly targeted variant analysis by different DNA amplification methods, including polymerase chain reaction (PCR), allele-specific PCR, PCR-single strand conformation polymorphism (PCR-SSCP), denaturing HPLC (DHPLC), TaqMan genotyping, direct (Sanger) sequencing, restriction fragment length polymorphism (RFLP), multiplex amplification refractory mutation (ARMS) and KASP genotyping (88). Some providers of clinical tests offer also larger sets of analyses, simultaneously detecting several different genetic polymorphisms. These panels include methodologies

such as microarrays, pyrosequencing, and next-generation sequencing (*e.g.,* massively parallel sequencing). As of now, the National Library of Medicine under the Genetic Testing Registry lists 71 centers, that provide genetic testing for thiopurines, that are located in the United States, Spain, Portugal, Germany, India, Turkey, Canada, Finland, Slovakia, Austria, Netherlands and South Korea (89).

Measurements of TPMT and NUDT15 activity

Numerous methods have been developed for the determination of TPMT activity. The enzymatic activity of TPMT can be monitored by detecting the formation of methylated products or through the consumption of the methyl donor SAM. The first step in TPMT activity assessment is the incubation of hemolysate or lysate of peripheral blood mononuclear cells with a substrate, 6-MP or 6-TG, and a methyl group donor, SAM. Upon incubation, a methylated product is formed and a methyl donor is consumed. The SAM consumed, or methylated products, 6-MMP or 6-MTG, can be detected in various ways. The enzyme activity is usually normalized and reported in terms of either hemoglobin content, packed red cells, erythrocyte count, or protein content.

The first methods for TPMT activity measurement evolved in the late 1970s and 1990s. These were radiochemical methods that measured the conversion of 6-MP to radioactively labeled 6-MMP, using C^{14}- or H^3-labelled-SAM (90–92). At the beginning of the 21st century, a rapid immunoassay for TPMT was invented, using an antibody specific for the methylated product, 6-MMP, that was attached to the sample well (Patent, WO 03/076894 A2). Another immunochemical assay was an ELISA-based assay for measuring TPMT activity (93). However, most methods currently used to assess TPMT activity use high-performance liquid chromatography (HPLC) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (58, 60, 94–97) The former methods exploit the absorbance of 6-MMP or the fluorescence of 6-MTG. LC-MS/MS methods have been developed to determine TPMT activity with high specificity and lower limits of detection as well as shorter analysis time (97). Specificities and sensitivities of TPMT activity tests to detect heterozygous or homozygous individuals range from 90.9–100.0 % and 13.4–100.0 %, resp. (88).

Thiopurine metabolite measurements

The metabolism of 6-MP is complex, involving numerous enzymes (8). This results in many metabolites, among which 6-TGNs and 6-MMP are the most significant. Monitoring the levels of these metabolites to enhance the efficacy of therapy and reduce adverse effects has been proven useful, especially in the treatment of autoimmune disorders (4, 98–100). Measuring the 6-TGNs and 6-MMP metabolites in erythrocytes facilitates dose adjustments for patients receiving the drug (32), as several clinical studies demonstrated a correlation between the levels of 6-TGN and the efficacy of therapy, as well as a correlation between the levels of 6-MMP and certain toxic effects (2, 13, 34–36).

Measurement of intracellular levels of thiopurine nucleotides in erythrocytes is usually assessed by chromatographic methods. Most of the analytical methods measure thiopurine metabolites in erythrocytes (101–103); however, methods using whole blood (104) and a dry blood spot (105) were also developed. The hemolysates from whole blood samples should be prepared as soon as possible, due to limited stability, especially of 6-TGN

(105–108). The stability of thiopurine metabolites is enhanced if the blood is spotted on appropriate filter papers and dried out completely (105).

After preparation, samples are separated and thiopurines are detected using HPLC- or UPLC-MS/MS methods (101–105, 109). The HPLC-UV method, compared to the LC-MS/MS method, is less expensive but requires more time for analysis and uses larger quantities of the mobile phase. The advantages of the LC-MS/MS method include shorter time of sample analysis and improved specificity, accuracy, and sensitivity (44).

FUTURE PERSPECTIVES

All three approaches, pharmacogenetics, phenotyping, and therapeutic drug monitoring, are complementary and exhibit great potential to significantly improve patient care by predicting and preventing adverse drug reactions. However, the extent of the implementation of different strategies for individualization of thiopurine dosing varies between different regions and different medical specialties and is difficult to estimate due to the lack of studies. It ranges from 67 % in the UK to 43 % globally (110), from 94 % in dermatology, 50 % in gastroenterology to 20 % in other immune-related conditions (110, 111). Some studies report that the patients are more likely to have their TPMT phenotype determined, compared to the TPMT genotype (112). However, due to the lack of studies, it is hard to define the exact extent of the use of TPMT activity measurements.

A recent systematic review of the cost-effectiveness of pharmacogenomics found that of 11 studies examining the pharmacoeconomic impact of these tests, eight demonstrated cost-effectiveness or even cost-savings associated with pharmacogenomic testing in thiopurine therapy (113). However, to improve the implementation of *TPMT* and *NUDT15* testing prior to treatment initiation, evidently several challenges need to be overcome. These include the need for multidisciplinary teams consisting of physicians, pharmacists, and other healthcare professionals to work together, all of whom need to be trained in the pharmacogenomic intricacies. In addition, the integration of pharmacogenomic data into electronic health records and decision support systems is essential for an efficient workflow but requires significant resources. The issue of reimbursement and the high costs associated with genetic testing are significant barriers, especially in less affluent regions. Finally, standardization is critical to ensure consistent and reliable pharmacogenomic practices.

Demand for population-specific guidelines

The need for population-specific pharmacogenomic guidelines for thiopurine treatment is particularly emphasized by the different allele frequencies of genetic polymorphisms in genes such as *TPMT* and *NUDT15* in different populations (Table II). The prevalence of *TPMT* and *NUDT15* polymorphisms affecting thiopurine metabolism and toxicity varies widely among Caucasians, Asians, and Africans (114, 115). According to guidelines, *NUDT15* genotyping is more relevant for individuals of East Asian and Hispanic descent with Native American ancestry, whereas *TPMT* genotyping is important among European and African populations (66).

Additionally, certain populations may be under-researched, so we may lack information on important genetic variants that influence the efficacy of thiopurines and the risk of adverse effects. Consequently, research findings from one population may not be

Table II. Frequencies of TPMT and NUDT15 variant alleles in biogeographical groups, based on joint consensus of AMP, CPIC, CAP, DPWG, ESPPT, PharmGKB for Tier 1 (TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C, NUDT15*3) and Tier 2 (TPMT*11, TPMT*29, TPMT*42, NUDT15*2, NUDT15*4, NUDT15*6, NUDT15*9, NUDT15*14) (75, 77, 116, 117)

Gene	Allele	Clinical function	Nucleotide change	Amino acid change	Common population frequencies
TIER1					
TPMT	*TPMT*2* *rs1800462*	No function	c.238G>C	p.Ala80Pro	**European: 0.24 %** African: 0.04 % East Asian: 0.01 % Central/South Asian: 0.00 % **Latino: 0.41 %**
	TPMT*3A *rs1800460* *rs1142345*	No function	c.460G>A and c.719A>G	p.Ala154Thr and p.Tyr240Cys	**European: 3.82 %** African: 0.65 % East Asian:0.07 % Central/South Asian: 0.47 % **Latino: 3.93 %**
	TPMT*3B *rs1800460*	No function	c.460G>A	p.Ala154Thr	European: 0.00 % African: 0.00 % East Asian: 0.00 % Central/South Asian: 0.00 % Latino: 0.01 %
	TPMT*3C *rs1142345*	No function	c.719A>G	p.Tyr240Cys	European: 0.43 % **African: 4.84 %** **East Asian: 1.99 %** **Central/South Asian: 1.09 %** Latino: 0.89 %
NUDT15	*NUDT15*3*	No function	c.415C>T	p.Arg139Cys	European: 0.30 % African: 0.06 % **East Asian: 5.81 %** **Central/South Asian: 6.17 %** Latino: 0.36 %
TIER 2					
TPMT	*TPMT*11* *rs72552738*	Decreased function	719A>G	Asn240Asp	European: < 0.01 % African: 0.00 % East Asian: 0.00 % Central/South Asian: 0.00 % Latino: 0.00 %
	TPMT*29 *rs267607275*	Decreased function	719A>G	Asn240Asp	European: 0.00 % African: 0.00 % East Asian: 0.01 % Central/South Asian: < 0.01 % Latino: 0.00 %
	TPMT*42 *rs759836180*	Decreased function	719A>G	Asn240Asp	European: < 0.01 % African: 0.00 % East Asian: 0.00 % Central/South Asian: 0.00 % Latino: 0.00 %

Table II. Continued

Gene	Allele	Clinical function	Nucleotide change	Amino acid change	Common population frequencies
NUDT15	NUDT15*2	No function	c.415C>T	p.Arg139Cys	European: 0.01 % African: 0.05 % **East Asian: 3.6 %** Central/South Asian: 0.06 % **Latino: 3.76 %**
	NUDT15*4	Uncertain function	c.416G>A	p.Arg139His	European: 0.01 % African: 0.03 % East Asian: 0.08 % Central/South Asian: 0.02 % **Latino: 1.25 %**
	NUDT15*6	Uncertain function	c.50_55dup	p.Gly17_ Val18dup	European: 0.28 % African: 0.21 % East Asian: 1.08 % Central/South Asian: 0.13 % Latino: 0.14 %
	NUDT15*9	No function	c.50_55del	p.Gly17_ Val18del	European: 0.2 % African: 0.03 % East Asian: 0.00 % Central/South Asian: 0.04 % Latino: 0.08 %
	NUDT15*14	Not assigned	415C>T	Arg139Cys	European: 0.01 % African: 0.00 % East Asian: 0.00 % Central/South Asian: 0.00 % Latino: 0.00 %

NUDT15 – nudix hydroase 15, *TPMT* – thiopurine *S*-methyltransferase; **bold** – populations with the allele frequency higher than 1 %.

directly applicable to another, underscoring the importance of complementary studies and the development of tailored pharmacogenomic guidelines to optimize therapeutic outcomes, minimize toxicity, and improve the efficacy of thiopurine therapy.

Emerging research in the field of thiopurine pharmacogenomics

The genetic polymorphisms in *TPMT* and *NUDT15* listed in Table I are the only variants clinically annotated with the highest level of evidence for pharmacogenetic testing (11, 77). However, the incomplete concordance between *TPMT* and *NUDT15* genotype or activity and response to thiopurines made it necessary to expand the portfolio of new biomarkers that could be used for individualization of thiopurine therapy (60). It was recognized that other genetic traits, such as genetic polymorphisms in *PACSIN2, ITPA, ABCC4, MTHFR, NT5C2, SLC291A,* and *SLC28A3*, influence the efficacy and safety of thiopurines.

PACSIN2 encodes a protein involved in linking the actin cytoskeleton to vesicle formation by regulating tubulin polymerization (118) and regulation of the migration behavior of malignant cells (119). In ALL patients, the homozygous genotype (TT) of the variant

rs2413739 (C>T) in the intronic region of *PACSIN2* was associated with lower TPMT activity compared to patients without this change (120, 121). The modulation of TPMT activity by PACSIN2 has also been validated in *in vitro* studies (120). The TT genotype for rs2413739 in *PACSIN2* has been associated with thiopurine-related hematotoxicity, hepatotoxicity, and gastrointestinal toxicity, particularly in patients with ALL (120–122); however, the mechanism of interaction between PACSIN2 and TPMT is not yet fully understood.

The nucleotidase NT5C2 dephosphorylates purine monophosphate nucleotides and is important for cellular nucleic acid homeostasis. The *NT5C2* variant has been identified as one of the most common genomic lesions specific for relapsed ALL and has been directly linked to thiopurine resistance (123–126). Germline mutations (rs72846714) (124, 125) and acquired somatic mutations (127) were associated with an increased incidence of relapse in ALL patients.

Another candidate considered as a potential pharmacogenetic marker that may influence metabolism and 6-MP-induced toxicities is inosine triphosphate pyrophosphatase (ITPA); however, results correlating its genetic polymorphisms with thiopurine outcome in ALL or IBD patients are inconsistent. The results of a recent meta-analysis examining ITPA polymorphisms and response to thiopurine in ALL patients show that *ITPA* 94C>A (rs1127354) is associated with 6-MP-induced neutropenia and hepatotoxicity, but not leukopenia (128). Results in IBD study cohorts are even more inconclusive, with many studies, including meta-analyses, finding no association between *ITPA* genotype and the development of thiopurine toxicity (129–133). Due to the involvement of the enzyme in thiopurine metabolism, certain *ITPA* variants (rs6139036, rs1127354) have been reported to affect the metabolic profile of thiopurines (134, 135). The differences in the results of these studies may arise from the different settings of the studies, including population characteristics (130, 131, 133, 136, 137) and the co-occurrence of polymorphisms in *TPMT* and *NUDT15* (138). It has been suggested that the *ITPA* genotype could serve as a genetic marker for improving risk stratification and therapy individualization in patients with ALL (139).

In addition to genetic biomarkers, metabolic factors have been shown to regulate TPMT activity and consequently could influence thiopurine treatment. Besides its methylation function, SAM plays a crucial role in stabilizing enzymes, and one of them is TPMT (140, 141). In experimental assays, the supplementation with SAM stabilizes TPMT posttranslationally and prevents degradation of variant TPMT (49, 141). Consequently, cells, such as MOLT lymphoblasts, cultured under conditions without SAM show significantly lower TPMT activity (142). Our previous findings further show, that TPMT activity measured in hemolysates positively correlates with the concentration of this cofactor (49, 50). Therefore, the measurement of SAM could contribute to the fine-tuning of thiopurine dosing.

Increased toxicity of thiopurines in patients with ALL has also been associated with polymorphisms that reduce the activity of methylenetetrahydrofolate reductase (MTHFR) (71). Considering the significant involvement of this enzyme in the formation and regulation of SAM, this information indicates that folate metabolism is a potential factor influencing the outcome of thiopurine treatment. The involvement of the folate cycle in TPMT activity and subsequently in the response to thiopurines has also been suggested by genetic polymorphisms in the thymidylate synthase (*TYMS*) gene (143). These polymorphisms may not only affect TPMT activity but have also been significantly associated with an increased risk of developing lymphopenia in patients with inflammatory bowel disease treated with thiopurines (144).

New technological advances in genetic testing

Recent advances in genetic testing technologies will have a significant impact on pharmacogenomics. The transition from targeted variant analysis to comprehensive pharmacogenomic panels has already enabled the simultaneous examination of multiple genetic markers in different genes, supporting precise drug therapy. Genes involved in the thiopurine response, in particular *TPMT, NUDT15*, and *NT5C2*, are included in several pharmacogenomic panels as well as panels for hematologic and solid tumor and cancer genomic profiling offered by different providers of genetic testing around the world (89, 145). The introduction of pharmacogenomic panels (passports) can greatly facilitate personalized medicine and adjust drug selection and dosing to optimize therapeutic outcomes and minimize adverse effects (146, 147). However, the clinical implementation of pharmacogenomics is fraught with challenges, including the complexity of interpreting extensive genetic data, the need for robust clinical guidelines to use this information effectively, concerns about the privacy and security of sensitive genetic information, and regulatory and reimbursement considerations (144, 148, 149). Improvements in analytical methods, software development, and artificial intelligence are likely to increase the functionality and consequently the use of such panels to better and more easily predict drug response (150).

Integrated approaches

Ideally, a futuristic projection on the future prospects of thiopurine therapy would involve an integrative approach in pharmacogenomics that comprehensively brings together multiple factors to personalize thiopurine dose adjustment. In addition to genetic variations in *TPMT, NUDT15*, and other genes mentioned above, these approaches would include environmental factors, patient characteristics such as age and comorbidities, co-therapy, and the metabolomic profile that includes thiopurine metabolite levels and other biochemical markers such as SAM and possibly folate cycle metabolites. Specific emphasis should be given to drug-drug interactions, as complex thiopurine metabolic pathways offer many targets for concomitantly used drugs. Among them, non-steroid anti-inflammatory drugs, furosemide, and sulfasalazine were reported to even inhibit TPMT (151–153). One of the most studied examples of interaction with thiopurines is allopurinol, which enhances thiopurine efficacy and is already applied together with thiopurines to treat refractory examples of IBD and ALL (154–156). The integration of these various data can be facilitated by sophisticated algorithms or predictive models that summarize and analyze the information to recommend optimized dosing regimens. Steps have already been taken toward such predictive models (157–160); however, the cost-effectiveness of such approaches has to be evaluated.

CONCLUSIONS

Pharmacogenomics and therapeutic drug monitoring (TDM) have greatly improved patient care in thiopurine therapy, addressing individual variability in efficacy and safety. Pharmacogenomics guidelines in personalized dosing by identifying *TPMT* and *NUDT15* genetic variants reduce adverse reactions and optimize outcomes of thiopurine therapy. Risk prediction *via* pharmacogenomics and enzyme activity assessment enables proactive

measures to prevent adverse drug reactions, while TDM ensures optimal drug levels, adjusting dosages based on individual factors. Emerging research in thiopurine pharmacogenomics, together with novel technological approaches in genetic testing, forecasts a new era of precision medicine where treatment is increasingly improved by the comprehensive integration of different data points. As the field continues to evolve, it is crucial to include these innovations, translating the knowledge into actionable strategies that refine patient treatment and elevate the standard of care in medical settings where thiopurines play a critical role.

Acronyms, abbreviations, symbols. – 6-MP – 6-mercaptopurine, 6-MMP – 6-methylmercaptopurine, 6-TdGTP – 6-thiodeoxyguanosine triphosphate, 6-TG – 6-thioguanine, 6-TGN – 6-thioguanine nucleotides, ALL – acute lymphoblastic leukemia, ARMS – multiplex amplification refractory mutation, AZA – azathioprine, CPIC – Clinical Pharmacogenetics Implementation Consortium, CPNDS – Canadian Pharmacogenomics Network for Drug Safety, DHPLC – denaturing HPLC, DPWG – Dutch Pharmacogenetics Working Group, GMPS – guanosine 5'-monophosphate synthase, HPLC – high-performance liquid chromatography, HPRT – hypoxanthine phosphoribosyltransferase, IBD – inflammatory bowel disease, IMPDH – inosine 5'-monophosphate dehydrogenase, ITPA – inosine triphosphate pyrophosphatase, LC-MS/MS – liquid chromatography coupled with tandem mass spectrometry, MTHFR – methylenetetrahydrofolate reductase, MTIMP – 6-methylthioinosine monophosphate, NT5C – nucleotidase, NUDT15 – nudix hydrolase 15, PCR – polymerase chain reaction, PCR-SSCP – PCR-single strand conformation polymorphism, RFLP – restriction fragment length polymorphism, SAM – S-adenosyl methionine, SNP – single nucleotide polymorphism, TDM – therapeutic drug monitoring, TGMP – 6-thioguanosine monophosphate, TGTP – 6-thioguanosine triphosphate, TIMP – 6-thioinosine monophosphate, TPMT – thiopurine S-methyltransferase, TYMS – thymidylate synthase, VNTRs – variable number of tandem repeats, XO – xanthine oxidase

Conflicts of interest. – The authors declare no conflict of interest.

Authors contributions. – Conceptualization, D.U. and I.M.R.; writing, review and editing, D.U., F.P., A.Š. and I.M.R. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. G. H. Hitchings and G. B. Elion, The chemistry and biochemistry of purine analogs, *Ann. NY Acad. Sci.* **60**(2) (1954) 195–199; https://doi.org/10.1111/j.1749-6632.1954.tb40008.x

2. C. A. Lamb, N. A. Kennedy, T. Raine, P. A. Hendy, P. J. Smith, J. K. Limdi, Bu'Hussain Hayee, M. C. E. Lomer, G. C. Parkes, C. Selinger, K. J. Barrett, R. J. Davies, C. Bennett, S. Gittens, M. G. Dunlop, O. Faiz, A. Fraser, V. Garrick, P. D. Johnston, M. Parkes, J. Sanderson and H. Terry; IBD guidelines eDelphi consensus group; D. R. Gaya, T. H. Iqbal, S. A. Taylor, M. Smith, M. Brookes, R. Hansen and A. B. Hawthorne, British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults, *Gut* **68**(Suppl. 3) (2019) s1–s106; https://doi.org/10.1136/gutjnl-2019-318484

3. N. Toft, H. Birgens, J. Abrahamsson, L. Griškevičius, H. Hallböök, M. Heyman, T. W. Klausen, Ó. G. Jónsson, K. Palk, K. Pruunsild, P. Quist-Paulsen, G. Vaitkeviciene, K. Vettenranta, A. Åsberg, T. L. Frandsen, H. V. Marquart, H. O. Madsen, U. Norén-Nyström and K. Schmiegelow, Results of NOPHO ALL2008 treatment for patients aged 1-45 years with acute lymphoblastic leukemia, *Leukemia* **32** (2018) 606–615; https://doi.org/10.1038/leu.2017.265

4. A. Singh, R. Mahajan, S. Kedia, A. K. Dutta, A. Anand, C. N. Bernstein, D. Desai, C. G. Pai, G. Makharia, H. V. Tevethia, J. W. Mak, K. Kaur, K. Peddi, M. K. Ranjan, P. Arkkila, R. Kochhar, R.

Banerjee, S. K. Sinha, S. C. Ng, S. Hanauer, S. Verma, U. Dutta, V. Midha, V. Mehta, V. Ahuja and A. Sood, Use of thiopurines in inflammatory bowel disease: an update, *Intest. Res.* **20**(1) (2022) 11–30; https://doi.org/10.5217/ir.2020.00155

5. C. Dai, Y.-H. Huang and M. Jiang, Combination therapy in inflammatory bowel disease: Current evidence and perspectives, *Int. Immunopharmacol.* **114** (2023) Article ID 109545; https://doi.org/10.1016/j.intimp.2022.109545

6. K. Tominaga, T. Sugaya, T. Tanaka, M. Kanazawa, M. Iijima and A. Irisawa, Thiopurines: Recent topics and their role in the treatment of inflammatory bowel diseases, *Front. Pharmacol.* **11** (2021) Article ID 582291 (10 pages); https://doi.org/10.3389/fphar.2020.582291

7. D. Christophorou, N. Funakoshi, Y. Duny, J.-C. Valats, M. Bismuth, G. Pineton De Chambrun, J.-P. Daures and P. Blanc, Systematic review with meta-analysis: infliximab and immunosuppressant therapy vs. infliximab alone for active ulcerative colitis, *Aliment. Pharmacol. Ther.* **41**(7) (2015) 603–612; https://doi.org/10.1111/apt.13102

8. R. Franca, G. Zudeh, S. Pagarin, M. Rabusin, M. Lucafò, G. Stocco and G. Decorti, Pharmacogenetics of thiopurines, *Cancer Drug Resist.* **2** (2019) 256–270; https://doi.org/10.20517/cdr.2019.004

9. G. Zaza, M. Cheok, N. Krynetskaia, C. Thorn, G. Stocco, J. M. Hebert, H. McLeod, R. M. Weinshilboum, M. V. Relling, W. E. Evans, T. E. Klein and R. B. Altman, Thiopurine pathway, *Pharmacogenet. Genomics* **20**(9) (2010) 573–574; https://doi.org/10.1097/FPC.0b013e328334338f

10. L. Lennard, J. A. Van Loon, J. S. Lilleyman and R. M. Weinshilboum, Thiopurine pharmacogenetics in leukemia: correlation of erythrocyte thiopurine methyltransferase activity and 6-thioguanine nucleotide concentrations, *Clin. Pharmacol. Ther.* **41**(1) (1987) 18–25; https://doi.org/10.1038/clpt.1987.4

11. M. V. Relling, M. Schwab, M. Whirl-Carrillo, G. Suarez-Kurtz, C.-H. Pui, C. M. Stein, A. M. Moyer, W. E. Evans, T. E. Klein, F. G. Antillon-Klussmann, K. E. Caudle, M. Kato, A. E. J. Yeoh, K. Schmiegelow and J. J. Yang, Clinical pharmacogenetics implementation consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update, *Clin. Pharmacol. Ther.* **105**(5) (2019) 1095–1105; https://doi.org/10.1002/cpt.1304

12. M. V. Relling, E. E. Gardner, W. J. Sandborn, K. Schmiegelow, C.-H. Pui, S. W. Yee, C. M. Stein, M. Carrillo, W. E. Evans and T. E. Klein, Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing, *Clin. Pharmacol. Ther.* **89**(3) (2011) 387–391; https://doi.org/10.1038/clpt.2010.320

13. T. A. de Beaumais, S. Lorrain, N. Mamhoudi, M. Simonin, C. Martinez Vinson, Y. Medard, A. Petit and E. Jacqz-Aigrain, Key factors associated with 6-thioguanine and 6-methylmercaptopurine nucleotide concentrations in children treated by thiopurine for acute leukaemia and inflammatory bowel disease, *Br. J. Clin. Pharmacol.* **90**(1) (2024) 209–219; https://doi.org/10.1111/bcp.15894

14. L. N. Toksvang, S. H. R. Lee, J. J. Yang and K. Schmiegelow, Maintenance therapy for acute lymphoblastic leukemia: basic science and clinical translations, *Leukemia* **36** (2022) 1749–1758; https://doi.org/10.1038/s41375-022-01591-4

15. L. Mei, E. P. Ontiveros, E. A. Griffiths, J. E. Thompson, E. S. Wang and M. Wetzler, Pharmacogenetics predictive of response and toxicity in acute lymphoblastic leukemia therapy, *Blood Rev.* **29**(4) (2015) 243–249; https://doi.org/10.1016/j.blre.2015.01.001

16. L. Lennard, C. S. Cartwright, R. Wade and A. Vora, Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: the influence of thiopurine methyltransferase pharmacogenetics, *Br. J. Haematol.* **169**(2) (2015) 228–240; https://doi.org/10.1111/bjh.13240

17. J. E. Axelrad, A. Roy, G. Lawlor, B. Korelitz and S. Lichtiger, Thiopurines and inflammatory bowel disease: Current evidence and a historical perspective, *World J. Gastroenterol.* **22**(46) (2016) 10103–10117; https://doi.org/10.3748/wjg.v22.i46.10103

18. S. Izraeli, C. Shochat, N. Tal and I. Geron, Towards precision medicine in childhood leukemia – Insights from mutationally activated cytokine receptor pathways in acute lymphoblastic leukemia, *Cancer Lett.* **352**(1) (2014) 15–20; https://doi.org/10.1016/j.canlet.2014.02.009

19. G. W. Moran, M.-F. Dubeau, G. G. Kaplan, H. Yang, B. Eksteen, S. Ghosh and R. Panaccione, Clinical predictors of thiopurine-related adverse events in Crohn's disease, *World J. Gastroenterol.* **21**(25) (2015) 7795–7804; https://doi.org/10.3748/wjg.v21.i25.7795

20. M. C. Dubinsky, H. Yang, P. V. Hassard, E. G. Seidman, L. Y. Kam, M. T. Abreu, S. R. Targan and E. A. Vasiliauskas, 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease, *Gastroenterology* **122**(4) (2002) 904–915; https://doi.org/10.1053/gast.2002.32420

21. T. Moriyama, R. Nishii, V. Perez-Andreu, W. Yang, F. A. Klussmann, X. Zhao, T.-N. Lin, K. Hoshitsuki, J. Nersting, K. Kihira, U. Hofmann, Y. Komada, M. Kato, R. McCorkle, L. Li, K. Koh, C. R. Najera, S. K.-Y. Kham, T. Isobe, Z. Chen, E. K. Chiew, D. Bhojwani, C. Jeffries, Y. Lu, M. Schwab, H. Inaba, C. H. Pui, M. V. Relling, A. Manabe, H. Hori, K. Schmiegelow, A. E. Yeoh, W. E. Evans and J. J. Yang, NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity, *Nat. Genet.* **48** (2016) 367–373; https://doi.org/10.1038/ng.3508

22. M. H. Vogt, E. H. Stet, R. A. De Abreu, J. P. Bökkerink, L. H. Lambooy and F. J. Trijbels, The importance of methylthio-IMP for methylmercaptopurine ribonucleoside (Me-MPR) cytotoxicity in Molt F4 human malignant T-lymphoblasts, *Biochim. Biophys. Acta* **1181**(2) (1993) 189–194; https://doi.org/10.1016/0925-4439(93)90110-m

23. P. W. Allan and L. L. Bennett, 6-Methylthioguanylic acid, a metabolite of 6-thioguanine, *Biochem. Pharmacol.* **20**(4) (1971) 847–852; https://doi.org/10.1016/0006-2952(71)90046-3

24. L. Lennard, The clinical pharmacology of 6-mercaptopurine, *Eur. J. Clin. Pharmacol.* **43** (1992) 329–339; https://doi.org/10.1007/BF02220605

25. A. K. Fotoohi, S. A. Coulthard and F. Albertioni, Thiopurines: factors influencing toxicity and response, *Biochem. Pharmacol.* **79**(9) (2010) 1211–1220; https://doi.org/10.1016/j.bcp.2010.01.006

26. I. Tiede, G. Fritz, S. Strand, D. Poppe, R. Dvorsky, D. Strand, H. A. Lehr, S. Wirtz, C. Becker, R. Atreya, J. Mudter, K. Hildner, B. Bartsch, M. Holtmann, R. Blumberg, H. Walczak, H. Iven, P. R. Galle, M. R. Ahmadian and M. F. Neurath, CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes, *J. Clin. Invest.* **111**(8) (2003) 1133–1145; https://doi.org/10.1172/JCI16432

27. G. Stocco, M. Pelin, R. Franca, S. De Iudicibus, E. Cuzzoni, D. Favretto, S. Martelossi, A. Ventura and G. Decorti, Pharmacogenetics of azathioprine in inflammatory bowel disease: A role for glutathione-S-transferase?, *World J. Gastroenterol.* **20**(13) (2014) 3534–3541; https://doi.org/10.3748/wjg.v20.i13.3534

28. R. Goldberg and P. M. Irving, Toxicity and response to thiopurines in patients with inflammatory bowel disease, *Expert Rev. Gastroenterol. Hepatol.* **9**(7) (2015) 891–900; https://doi.org/10.1586/17474124.2015.1039987

29. A. M. Abbas, R. M. Almukhtar, E. V. Loftus, G. R. Lichtenstein and N. Khan, Risk of melanoma and non-melanoma skin cancer in ulcerative colitis patients treated with thiopurines: a nationwide retrospective cohort, *Am. J. Gastroenterol.* **109**(11) (2014) 1781–1793; https://doi.org/10.1038/ajg.2014.298

30. D. S. Kotlyar, J. D. Lewis, L. Beaugerie, A. Tierney, C. M. Brensinger, J. P. Gisbert, E. V. Loftus, L. Peyrin-Biroulet, W. C. Blonski, M. Van Domselaar, M. Chaparro, S. Sandilya, M. Bewtra, F. Beigel, L. Biancone and G. R. Lichtenstein, Risk of lymphoma in patients with inflammatory bowel disease treated with azathioprine and 6-mercaptopurine: A meta-analysis, *Clin. Gastroenterol. Hepatol.* **13**(5) (2015) 847–858; https://doi.org/10.1016/j.cgh.2014.05.015

31. P. Deepak and D. J. Stobaugh, Risk of myeloid neoplasms in inflammatory bowel disease patients is linked to exposure to thiopurines and not with tumor necrosis factor-alpha inhibitors, *Clin. Gastroenterol. Hepatol.* **13**(10) (2015) 1857–1858; https://doi.org/10.1016/j.cgh.2014.08.025

32. B. Warner, E. Johnston, M. Arenas-Hernandez, A. Marinaki, P. Irving and J. Sanderson, A practical guide to thiopurine prescribing and monitoring in IBD, *Frontline Gastroenterol.* **9** (2018) 10–15; https://doi.org/10.1136/flgastro-2016-100738

33. M. Campbell, C. Kiss, M. Zimmermann, C. Riccheri, J. Kowalczyk, M. S. Felice, M. Kuzmanovic, G. Kovacs, H. Kosmidis, A. Gonzalez, E. Bilic, L. Castillo, A. Kolenova, J. Jazbec, A. Popa, D. Konstantinov, J. Kappelmayer, T. Szczepanski, M. Dworzak, B. Buldini, G. Gaipa, N. Marinov, J. Rossi, A. Nagy, I. Gaspar, J. Stary and M. Schrappe, Childhood acute lymphoblastic leukemia: Results of the randomized acute lymphoblastic leukemia Intercontinental-Berlin-Frankfurt-Münster 2009 trial, *J. Clin. Oncol.* **41**(19) (2023) 3499–3511; https://doi.org/10.1200/JCO.22.01760

34. D. R. Wong, M. J. H. Coenen, S. H. Vermeulen, L. J. J. Derijks, C. J. van Marrewijk, O. H. Klungel, H. Scheffer, B. Franke, H.-J. Guchelaar, D. J. de Jong, L. G. J. B. Engels, A. L. M. Verbeek, P. M. Hooymans, and TOPIC recruitment team, Early assessment of thiopurine metabolites identifies patients at risk of thiopurine-induced leukopenia in inflammatory bowel disease, *J. Crohns Colitis* **11**(2) (2017) 175–184; https://doi.org/10.1093/ecco-jcc/jjw130

35. K. Keyashian, Monitoring and management of toxicities in long-term thiopurine therapy, *Gastroenterol. Hepatol.* (NY) **9**(10) (2013) 672–674.

36. K. Bradford and D. Q. Shih, Optimizing 6-mercaptopurine and azathioprine therapy in the management of inflammatory bowel disease, *World J. Gastroenterol.* **17**(37) (2011) 4166–4173; https://doi.org/10.3748/wjg.v17.i37.4166

37. M. C. Dubinsky, S. Lamothe, H. Y. Yang, S. R. Targan, D. Sinnett, Y. Théorêt and E. G. Seidman, Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease, *Gastroenterology* **118**(4) (2000) 705–713; https://doi.org/10.1016/s0016-5085(00)70140-5

38. P. Brown, H. Inaba, C. Annesley, J. Beck, S. Colace, M. Dallas, K. DeSantes, K. Kelly, C. Kitko, N. Lacayo, N. Larrier, L. Maese, K. Mahadeo, R. Nanda, V. Nardi, V. Rodriguez, J. Rossoff, L. Schuettpelz, L. Silverman, J. Sun, W. Sun, D. Teachey, V. Wong, G. Yanik, A. Johnson-Chilla and N. Ogba, Pediatric acute lymphoblastic leukemia, Version 2.2020, NCCN Clinical practice guidelines in oncology, *J. Nat. Compr. Cancer Network* **18**(1) (2020) 81–112; https://doi.org/10.6004/jnccn.2020.0001

39. C. Cuffari, Y. Théorêt, S. Latour and G. Seidman, 6-Mercaptopurine metabolism in Crohn's disease: correlation with efficacy and toxicity, *Gut* **39** (1996) 401–406; https://doi.org/10.1136/gut.39.3.401

40. L. J. J. Derijks, L. P. L. Gilissen, P. M. Hooymans and D. W. Hommes, Review article: thiopurines in inflammatory bowel disease, *Alim. Pharmacol. Therap.* **24**(5) (2006) 715–729; https://doi.org/10.1111/j.1365-2036.2006.02980.x

41. H. E. Mardini and G. L. Arnold, Utility of measuring 6-methylmercaptopurine and 6-thioguanine nucleotide levels in managing inflammatory bowel disease patients treated with 6-mercaptopurine in a clinical practice setting, *J. Clin. Gastroenterol.* **36**(5) (2003) 390–395; https://doi.org/10.1097/00004836-200305000-00005

42. D. P. van Asseldonk, M. L. Seinen, N. K. H. de Boer, A. A. van Bodegraven and C. J. Mulder, Hepatotoxicity associated with 6-methyl mercaptopurine formation during azathioprine and 6-mercaptopurine therapy does not occur on the short-term during 6-thioguanine therapy in IBD treatment, *J. Crohn's Colitis* **6**(1) (2012) 95–101; https://doi.org/10.1016/j.crohns.2011.07.009

43. J. P. Gisbert, Y. González-Lama and J. Maté, Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review, *Am. J. Gastroenterol.* **102**(7) (2007) 1518–1527; https://doi.org/10.1111/j.1572-0241.2007.01187.x

44. A. F. Y. A. Hadithy, N. K. H. de Boer, L. J. J. Derijks, J. C. Escher, C. J. J. Mulder and J. R. B. J. Brouwers, Thiopurines in inflammatory bowel disease: pharmacogenetics, therapeutic drug monitoring and clinical recommendations, *Dig. Liver Dis.* **37**(4) (2005) 282–297; https://doi.org/10.1016/j.dld.2004.09.029

45. L. Lennard, C. S. Cartwright, R. Wade and A. Vora, Thiopurine methyltransferase and treatment outcome in the UK acute lymphoblastic leukaemia trial ALL2003, *Br. J. Haematol.* **170**(4) (2015) 550–558; https://doi.org/10.1111/bjh.13469

46. C. N. Remy, Metabolism of thiopyrimidines and thiopurines. S-Methylation with S-adenosylmethionine transmethylase and catabolism in mammalian tissues, *J. Biol. Chem.* **238**(3) (1963) 1078–1084.

47. L. C. Woodson, M. M. Ames, C. D. Selassie, C. Hansch and R. M. Weinshilboum, Thiopurine methyltransferase. Aromatic thiol substrates and inhibition by benzoic acid derivatives, *Mol. Pharmacol.* **24**(3) (1983) 471–478.

48. Y. Peng, Q. Feng, D. Wilk, A. A. Adjei, O. E. Salavaggione, R. M. Weinshilboum and V. C. Yee, Structural basis of substrate recognition in thiopurine s-methyltransferase, *Biochemistry* **47**(23) (2008) 6216–6225; https://doi.org/10.1021/bi800102x

49. M. Milek, A. Smid, R. Tamm, N. K. Kuzelicki, A. Metspalu and I. Mlinaric-Rascan, Post-translational stabilization of thiopurine S-methyltransferase by S-adenosyl-L-methionine reveals regulation of TPMT*1 and *3C allozymes, *Biochem. Pharmacol.* **83**(7) (2012) 969–976; https://doi.org/10.1016/j.bcp.2012.01.010

50. N. Karas-Kuželički, A. Šmid, R. Tamm, A. Metspalu and I. Mlinarič-Raščan, From pharmacogenetics to pharmacometabolomics: SAM modulates TPMT activity, *Pharmacogenomics* **15**(11) (2014) 1437–1449; https://doi.org/10.2217/pgs.14.84

51. NIH – NLM – NCBI, TPMT thiopurine S-methyltransferase [*Homo sapiens* (human)] – Gene ID: 7172, updated on 17-Jun-2024; https://www.ncbi.nlm.nih.gov/gene/7172, last acces date August 4, 2024.

52. M. L. Appell, J. Berg, J. Duley, W. E. Evans, M. A. Kennedy, L. Lennard, T. Marinaki, H. L. McLeod, M. V. Relling, E. Schaeffeler, M. Schwab, R. Weinshilboum, A. E. J. Yeoh, E. M. McDonagh, J. M. Hebert, T. E. Klein and S. A. Coulthard, Nomenclature for alleles of the thiopurine methyltransferase gene, *Pharmacogenet. Genomics* **23**(4) (2013) 242–248; https://doi.org/10.1097/FPC.0b013e32835f1cc0

53. Linköping University, TPMT nomenclature committee (TPMT Alleles); LiU, Linköping (Sweden), Nov 2022; https://liu.se/en/research/tpmt-nomenclature-committee, last access date August 4, 2024.

54. E. Y. Krynetski, M. Y. Fessing, C. R. Yates, D. Sun, J. D. Schuetz and W. E. Evans, Promoter and intronic sequences of the human thiopurine S-methyltransferase (TPMT) gene isolated from a human pacl genomic library, *Pharm. Res.* **14** (1997) 1672–1678; https://doi.org/10.1023/A:1012111325397

55. E. Y. Krynetski, N. F. Krynetskaia, Y. Yanishevski and W. E. Evans, Methylation of mercaptopurine, thioguanine, and their nucleotide metabolites by heterologously expressed human thiopurine S-methyltransferase, *Mol. Pharmacol.* **47**(6) (1995) 1141–1147.

56. C. Szumlanski, D. Otterness, C. Her, D. Lee, B. Brandriff, D. Kelsell, N. Spurr, L. Lennard, E. Wieben and R. Weinshilboum, Thiopurine methyltransferase pharmacogenetics: human gene cloning and characterization of a common polymorphism, *DNA Cell Biol.* **15**(1) (1996) 17–30; https://doi.org/10.1089/dna.1996.15.17

57. H. L. Tai, E. Y. Krynetski, C. R. Yates, T. Loennechen, M. Y. Fessing, N. F. Krynetskaia and W. E. Evans, Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians, *Am. J. Human Genet.* **58**(4) (1996) 694–702.

58. M. Milek, J. Murn, Z. Jaksic, J. Lukac Bajalo, J. Jazbec and I. Mlinaric Rascan, Thiopurine S-methyltransferase pharmacogenetics: genotype to phenotype correlation in the Slovenian population, *Pharmacology* **77**(3) (2006) 105–114; https://doi.org/10.1159/000093278

59. E. Krynetski and W. E. Evans, Drug methylation in cancer therapy: lessons from the TPMT polymorphism, *Oncogene* **22** (2003) 7403–7413; https://doi.org/10.1038/sj.onc.1206944

60. L. Lennard, C. S. Cartwright, R. Wade, S. M. Richards and A. Vora, Thiopurine methyltransferase genotype-phenotype discordance and thiopurine active metabolite formation in childhood acute lymphoblastic leukaemia, *Br. J. Clin. Pharmacol.* **76**(1) (2013) 125–136; https://doi.org/10.1111/bcp.12066

61. A. G. McLennan, The Nudix hydrolase superfamily, *Cell Mol. Life Sci.* **63** (2006) 123–143; https://doi.org/10.1007/s00018-005-5386-7

62. M. Carter, A.-S. Jemth, A. Hagenkort, B. D. G. Page, R. Gustafsson, J. J. Griese, H. Gad, N. C. K. Valerie, M. Desroses, J. Boström, U. Warpman Berglund, T. Helleday and P. Stenmark, Crystal structure, biochemical and cellular activities demonstrate separate functions of MTH1 and MTH2, *Nat. Commun.* **6** (2015) Article ID 7871 (10 pages); https://doi.org/10.1038/ncomms8871

63. NIH – NLM – NCBI, NUDT15 nudix hydrolase 15 [*Homo sapiens* (human)] – Gene ID: 55270, updated on 8-Jul-2024; https://www.ncbi.nlm.nih.gov/gene/55270; last access date August 4, 2024.

64. S.-K. Yang, M. Hong, J. Baek, H. Choi, W. Zhao, Y. Jung, T. Haritunians, B. D. Ye, K.-J. Kim, S. H. Park, S.-K. Park, D.-H. Yang, M. Dubinsky, I. Lee, D. P. B. McGovern, J. Liu and K. Song, A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia, *Nat. Genet.* **46** (2014) 1017–1020; https://doi.org/10.1038/ng.3060

65. D. Yin, X. Xia, J. Zhang, S. Zhang, F. Liao, G. Zhang, Y. Zhang, Q. Hou, X. Yang, H. Wang, Z. Ma, H. Wang, Y. Zhu, W. Zhang, Y. Wang, B. Liu, L. Wang, H. Xu and Y. Shu, Impact of NUDT15 polymorphisms on thiopurines-induced myelotoxicity and thiopurines tolerance dose, *Oncotarget* **8** (2017) 13575–13585; https://doi.org/10.18632/oncotarget.14594

66. J. J. Yang, W. Landier, W. Yang, C. Liu, L. Hageman, C. Cheng, D. Pei, Y. Chen, K. R. Crews, N. Kornegay, F. L. Wong, W. E. Evans, C.-H. Pui, S. Bhatia and M. V. Relling, Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia, *J. Clin. Oncol.* **33** (2015) 1235–1242; https://doi.org/10.1200/JCO.2014.59.4671

67. A. Gaedigk, S. T. Casey, M. Whirl-Carrillo, N. A. Miller and T. E. Klein, Pharmacogene variation consortium: a global resource and repository for pharmacogene variation, *Clin. Pharmacol. Ther.* **110**(3) (2021) 542–545; https://doi.org/10.1002/cpt.2321

68. J. J. Yang, M. Whirl-Carrillo, S. A. Scott, A. J. Turner, M. Schwab, Y. Tanaka, G. Suarez-Kurtz, E. Schaeffeler, T. E. Klein, N. A. Miller and A. Gaedigk, Pharmacogene variation consortium gene introduction: NUDT15, *Clin. Pharmacol. Therap.* **105**(5) (2019) 1091–1094; https://doi.org/10.1002/cpt.1268

69. E. Schaeffeler, C. Fischer, D. Brockmeier, D. Wernet, K. Moerike, M. Eichelbaum, U. M. Zanger and M. Schwab, Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants, *Pharmacogenetics* **14**(7) (2004) 407–417; https://doi.org/10.1097/01.fpc.0000114745.08559.db

70. M. Stanulla, E. Schaeffeler, T. Flohr, G. Cario, A. Schrauder, M. Zimmermann, K. Welte, W.-D. Ludwig, C. R. Bartram, U. M. Zanger, M. Eichelbaum, M. Schrappe and M. Schwab, Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia, *JAMA* **293**(12) (2005) 1485–1489; https://doi.org/10.1001/jama.293.12.1485

71. N. Karas-Kuzelicki, J. Jazbec, M. Milek and I. Mlinaric-Rascan, Heterozygosity at the TPMT gene locus, augmented by mutated MTHFR gene, predisposes to 6-MP related toxicities in childhood ALL patients, *Leukemia* **23** (2009) 971–974; https://doi.org/10.1038/leu.2008.317

72. E. Schaeffeler, S. U. Jaeger, V. Klumpp, J. J. Yang, S. Igel, L. Hinze, M. Stanulla and M. Schwab, Impact of *NUDT15* genetics on severe thiopurine-related hematotoxicity in patients with European ancestry, *Gen. Med.* **21**(9) (2019) 2145–2150; https://doi.org/10.1038/s41436-019-0448-7

73. L. Dean and M. Kane, *Mercaptopurine Therapy and* TPMT *and* NUDT15 *Genotype*, in *Medical Genetics Summaries* (Eds. V. M. Pratt, S. A. Scott, M. Pirmohamed, B. Esquivel, B. L. Kattman and A. J. Malheiro); National Center for Biotechnology Information (US), Bethesda (MD), 2012; https://www.ncbi.nlm.nih.gov/books/NBK100660/

74. L. L. Goh, C. W. Lim, K. P. Leong and K. H. Ong, TPMT and NUDT15 testing for thiopurine therapy: A major tertiary hospital experience and lessons learned, *Front. Pharmacol.* **13** (2022) Article ID 837164 (10 pages); https://doi.org/10.3389/fphar.2022.837164

75. M. Whirl-Carrillo, R. Huddart, L. Gong, K. Sangkuhl, C. F. Thorn, R. Whaley and T. E. Klein, An evidence-based framework for evaluating pharmacogenomics knowledge for personalized medicine, *Clin. Pharmacol. Ther.* **110**(3) (2021) 563–572; https://doi.org/10.1002/cpt.2350

76. C. J. D. Ross, H. Visscher, J. Sistonen, L. R. Brunham, K. Pussegoda, T. T. Loo, M. J. Rieder, G. Koren, B. C. Carleton, M. R. Hayden, and CPNDS Consortium, The Canadian Pharmacogenomics Network for Drug Safety: A model for safety pharmacology, *Thyroid* **20**(7) (2010) 681–687; https://doi.org/10.1089/thy.2010.1642

77. V. M. Pratt, L. H. Cavallari, M. L. Fulmer, A. Gaedigk, H. Hachad, Y. Ji, L. V. Kalman, R. C. Ly, A. M. Moyer, S. A. Scott, R. H. N. van Schaik, M. Whirl-Carrillo and K. E. Weck, TPMT and NUDT15 genotyping recommendations: A joint consensus recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase, *J. Mol. Diagn.* **24**(10) (2022) 1051–1063; https://doi.org/10.1016/j.jmoldx.2022.06.007

78. PharmGKB, *Azathioprine*; https://www.pharmgkb.org/chemical/PA448515/prescribingInfo, last access date August 4, 2024.

79. PharmGKB, *Mercaptopurine*; https://www.pharmgkb.org/chemical/PA450379/prescribing Info, last access date August 4, 2024.

80. PharmGKB, *Thioguanine*; https://www.pharmgkb.org/chemical/PA451663/prescribingInfo, last access date August 4, 2024.

81. M. Maillard, R. Nishii, W. Yang, K. Hoshitsuki, D. Chepyala, S. H. R. Lee, J. Q. Nguyen, M. V. Relling, K. R. Crews, M. Leggas, M. Singh, J. L. Y. Suang, A. E. J. Yeoh, S. Jeha, H. Inaba, C.-H. Pui, S. E. Karol, A. Trehan, P. Bhatia, F. G. Antillon Klussmann, D. Bhojwani, C. E. Haidar and J. J. Yang, Additive effects of TPMT and NUDT15 on thiopurine toxicity in children with acute lymphoblastic leukemia across multiethnic populations, *J. Natl. Cancer Inst.* **116**(5) (2024) 702–710; https://doi.org/10.1093/jnci/djae004

82. T. R. Yakushi, Y. Qu, M. M. Moradian and R. T. Ramjit, Comprehensive evaluation of how TPMT genotype influences thiopurine treatment, *Transl. Biomed.* **13**(12) (2022) 1–11; https://doi.org/10.36648/2172

83. D. F. Carr, R. M. Turner and M. Pirmohamed, Pharmacogenomics of anticancer drugs: Personalising the choice and dose to manage drug response, *Br. J. Clin. Pharmacol.* **87**(2) (2021) 237–255; https://doi.org/10.1111/bcp.14407

84. D. Desai, A. Jena, V. Sharma and T. Hibi, Time to incorporate preemptive NUDT15 testing before starting thiopurines in inflammatory bowel disease in Asia and beyond: a review, *Expert Rev. Clin. Pharmacol.* **16** (2023) 643–653; https://doi.org/10.1080/17512433.2023.2232300

85. O. Dewit, T. Moreels, F. Baert, H. Peeters, C. Reenaers, M. de Vos, P. Van Hootegem, V. Muls, G. Veereman, F. Mana, M. Van Outryve, J. Holvoet, S. Naegels, H. Piessevaux, Y. Horsmans, J. L. Gala and Belgian Inflammatory Bowel Disease Research Group (BIRD), Limitations of extensive TPMT genotyping in the management of azathioprine-induced myelosuppression in IBD patients, *Clin. Biochem.* **44**(13) (2011) 1062–1066; https://doi.org/10.1016/j.clinbiochem.2011.06.079

86. K. Zarca, I. Durand-Zaleski, M.-A. Loriot, G. Chatellier and N. Pallet, Modeling the outcome of systematic TPMT genotyping or phenotyping before azathioprine prescription: A cost-effectiveness analysis, *Mol. Diagn. Ther.* **23** (2019) 429–438; https://doi.org/10.1007/s40291-019-00398-x

87. A. A. Omar, L. Basiouny, A. S. Elnoby, A. Zaki and M. Abouzid, St. Jude Total Therapy studies from I to XVII for childhood acute lymphoblastic leukemia: a brief review, *J. Egypt. Natl. Cancer Inst.* **34** (2022) Article ID 25 (20 pages); https://doi.org/10.1186/s43046-022-00126-3

88. L. M. Roy, R. M. Zur, E. Uleryk, C. Carew, S. Ito and W. J. Ungar, Thiopurine S-methyltransferase testing for averting drug toxicity in patients receiving thiopurines: a systematic review, *Pharmacogenomics* **17**(6) (2016) 633–656; https://doi.org/10.2217/pgs.16.12

89. NIH – NLM – NCBI, Genetic Testing Registry (GTR), *Clinical and Research Tests for Thiopurine*; https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=thiopurine; last access date August 4, 2024.

90. R. M. Weinshilboum, F. A. Raymond and P. A. Pazmiño, Human erythrocyte thiopurine methyl-transferase: radiochemical microassay and biochemical properties, *Clin. Chim. Acta* **85**(3) (1978) 323–333; https://doi.org/10.1016/0009-8981(78)90311-x

91. M. Tinel, A. Berson, D. Pessayre, P. Letteron, M. P. Cattoni, Y. Horsmans and D. Larrey, Pharmaco-genetics of human erythrocyte thiopurine methyltransferase activity in a French population, *Br. J. Clin. Pharmacol.* **32**(6) (1991) 729–734.

92. U. Hindorf and M. L. Appell, Genotyping should be considered the primary choice for pre-treatment evaluation of thiopurine methyltransferase function, *J. Crohns Colitis* **6**(6) (2012) 655–659; https://doi.org/10.1016/j.crohns.2011.11.014

93. T. Larussa, E. Suraci, M. Lentini, I. Nazionale, L. Gallo, L. Abenavoli, M. Imeneo, F. S. Costanzo, G. Cuda and F. Luzza, High prevalence of polymorphism and low activity of thiopurine methyltrans-ferase in patients with inflammatory bowel disease, *Eur. J. Intern. Med.* **23**(3) (2012) 273–277; https://doi.org/10.1016/j.ejim.2011.12.002

94. T. Kröplin, C. Fischer and H. Iven, Inhibition of thiopurine S-methyltransferase activity by impu-rities in commercially available substrates: a factor for differing results of TPMT measurements, *Eur. J. Clin. Pharmacol.* **55**(4) (1999) 285–291.

95. L. Ben Salah, M. Belkhiria el Haj Amor, C. Chbili, S. Khlifi, N. Fathallah, I. Bougmiza, E. Ben Jazia, N. Houdret, C. Ben Salem and S. Saguem, Analysis of thiopurine S-methyltransferase phenotype-genotype in a Tunisian population with Crohn's disease, *Eur. J. Drug Metab. Pharmacokin.* **38** (2013) 241–244; https://doi.org/10.1007/s13318-013-0127-z

96. R. Tamm, R. Mägi, R. Tremmel, S. Winter, E. Mihailov, A. Smid, A. Möricke, K. Klein, M. Schrappe, M. Stanulla, R. Houlston, R. Weinshilboum, I. Mlinarič Raščan, A. Metspalu, L. Milani, M. Schwab and E. Schaeffeler, Polymorphic variation in TPMT is the principal determinant of TPMT pheno-type: A meta-analysis of three genome-wide association studies, *Clin. Pharmacol. Ther.* **101**(5) (2017) 684–695; https://doi.org/10.1002/cpt.540

97. D. Pecher, S. Dokupilová, Z. Zelinková, M. Peppelenbosch, V. Mikušová and P. Mikuš, Determina-tion of thiopurine *S*-methyltransferase activity by hydrophilic interaction liquid chromatography hyphenated with mass spectrometry, *J. Pharm. Biomed. Anal.* **142** (2017) 244–251; https://doi.org/10.1016/j.jpba.2017.05.016

98. L. Wilson, S. Tuson, L. Yang and D. Loomes, Real-world use of azathioprine metabolites changes clinical management of inflammatory bowel disease, *J. Can. Assoc. Gastroenterol.* **4**(3) (2020) 101–109; https://doi.org/10.1093/jcag/gwaa005

99. Y. González-Lama and J. P. Gisbert, Monitoring thiopurine metabolites in inflammatory bowel disease, *Frontline Gastroenterol.* **7** (2016) 301–307; https://doi.org/10.1136/flgastro-2015-100681

100. P. Sousa, M. M. Estevinho, C. C. Dias, P. Ministro, U. Kopylov, S. Danese, L. Peyrin-Biroulet and F. Magro, Thiopurines' metabolites and drug toxicity: A meta-analysis, *J. Clin. Med.* **9**(7) (2020) Ar-ticle ID 2216 (29 pages); https://doi.org/10.3390/jcm9072216101

101. T. Dervieux and R. Boulieu, Simultaneous determination of 6-thioguanine and methyl 6-mercap-topurine nucleotides of azathioprine in red blood cells by HPLC, *Clin. Chem.* **44**(3) (1998) 551–555.

102. G. Cangemi, A. Barabino, S. Barco, A. Parodi, S. Arrigo and G. Melioli, A validated HPLC method for the monitoring of thiopurine metabolites in whole blood in paediatric patients with inflamma-tory bowel disease, *Int. J. Immunopathol. Pharmacol.* **25**(2) (2012) 435–444; https://doi.org/10.1177/039463201202500213

103. M. F. Neurath, R. Kiesslich, U. Teichgräber, C. Fischer, U. Hofmann, M. Eichelbaum, P. R. Galle and M. Schwab, 6-Thioguanosine diphosphate and triphosphate levels in red blood cells and response to azathioprine therapy in Crohn's Disease, *Clin. Gastroenterol. Hepatol.* **3**(10) (2005) 1007–1014; https://doi.org/10.1016/S1542-3565(05)00697-X

104. Q. Miao, Y.-J. Bai, J.-L. Zhang, Yi Li, Z.-Z. Su, L. Yan, L.-L. Wang and Y.-G. Zou, Highly sensitive and rapid determination of azathioprine metabolites in whole blood lysate by liquid chromatography-tandem mass spectrometry, *J. Chromatogr. B* **1136** (2020) Article ID 121802; https://doi.org/10.1016/j.jchromb.2019.121802

105. K. Lampič, J. Trontelj, H. Prosen, D. Drobne, A. Šmid and T. Vovk, Determination of 6-thioguanine and 6-methylmercaptopurine in dried blood spots using liquid chromatography-tandem mass spectrometry: Method development, validation and clinical application, *Clin. Chim. Acta* **499** (2019) 24–33; https://doi.org/10.1016/j.cca.2019.08.024

106. I.-Y. Yoo, K. Lee, O.-J. Ji, H. I. Woo and S.-Y. Lee, Evaluation of stability of thiopurine metabolites using a validated LC-MS/MS method, *Ann. Lab. Med.* **38**(3) (2018) 255–260; https://doi.org/10.3343/alm.2018.38.3.255

107. P. W. Lowry, C. L. Franklin, A. L. Weaver, M. G. Pike, D. C. Mays, W. J. Tremaine, J. J. Lipsky and W. J. Sandborn, Measurement of thiopurine methyltransferase activity and azathioprine metabolites in patients with inflammatory bowel disease, *Gut* **49** (2001) 665–670; https://doi.org/10.1136/gut.49.5.665

108. P. de Graaf, N. K. H. de Boer, B. Jharap, C. J. J. Mulder, A. A. van Bodegraven and A. I. Veldkamp, Stability of thiopurine metabolites: A potential analytical bias, *Clin. Chem.* **54**(1) (2008) 216–218; https://doi.org/10.1373/clinchem.2007.092676

109. H. Yu, D. Li, D. Xiang, X. Li, L. Liu, D. Liu and X. Gong, Development and validation of a novel HPLC-UV method for simultaneous determination of azathioprine metabolites in human red blood cells, *Heliyon* **9** (2023) Article ID e13870 (9 pages); https://doi.org/10.1016/j.heliyon.2023.e13870

110. L. Lennard, Implementation of TPMT testing, *Br. J. Clin. Pharmacol.* **77**(4) (2014) 704–714; https://doi.org/10.1111/bcp.12226

111. Y. Kakuta, M. Kato, Y. Shimoyama, T. Naito, R. Moroi, M. Kuroha, H. Shiga, Y. Kinouchi and A. Masamune, Usefulness and difficulties with the thiopurine pharmacogenomic *NUDT15* genotyping test: Analysis of real-world data in Japan, *J. Pharmacol. Sci.* **153**(3) (2023) 161–169; https://doi.org/10.1016/j.jphs.2023.09.002

112. K. W. Weitzel, D. M. Smith, A. R. Elsey, B. Q. Duong, B. Burkley, M. Clare-Salzler, Y. Gong, T. A. Higgins, B. Kong, T. Langaee, C. W. McDonough, B. J. Staley, T. T. Vo, D. T. Wake, L. H. Cavallari and J. A. Johnson, Implementation of standardized clinical processes for TPMT testing in a diverse multidisciplinary population: Challenges and lessons learned, *Clin. Trans. Sci.* **11**(2) (2018) 175–181; https://doi.org/10.1111/cts.12533

113. S. A. Morris, A. T. Alsaidi, A. Verbyla, A. Cruz, C. Macfarlane, J. Bauer and J. N. Patel, Cost effectiveness of pharmacogenetic testing for drugs with clinical pharmacogenetics implementation consortium (CPIC) guidelines: A systematic review, *Clin. Pharmacol. Therap.* **112**(6) (2022) 1318–1328; https://doi.org/10.1002/cpt.2754

114. G. Suarez-Kurtz, Population impact of pharmacogenetic tests in admixed populations across the Americas, *Pharmacogenomics J.* **21** (2021) 216–221; https://doi.org/10.1038/s41397-020-00200-w

115. Y. Zhou and V. M. Lauschke, Population pharmacogenomics: an update on ethnogeographic differences and opportunities for precision public health, *Hum. Genet.* **141** (2022) 1113–1136; https://doi.org/10.1007/s00439-021-02385-x

116. K. J. Karczewski, L. C. Francioli, G. Tiao, B. B. Cummings, J. Alföldi, Q. Wang, R. L. Collins, K. M. Laricchia, A. Ganna, D. P. Birnbaum, L. D. Gauthier, H. Brand, M. Solomonson, N. A. Watts, D. Rhodes, M. Singer-Berk, E. M. England, E. G. Seaby, J. A. Kosmicki, R. K. Walters, K. Tashman, Y. Farjoun, E. Banks, T. Poterba, A. Wang, C. Seed, N. Whiffin, J. X. Chong, K. E. Samocha, E. Pierce-Hoffman, Z. Zappala, A. H. O'Donnell-Luria, E. V. Minikel, B. Weisburd, M. Lek, J. S. Ware, C. Vittal, I. M. Armean, L. Bergelson, K. Cibulskis, K. M. Connolly, M. Covarrubias, S. Donnelly, S. Ferriera, S. Gabriel, J. Gentry, N. Gupta, T. Jeandet, D. Kaplan, C. Llanwarne, R. Munshi, S. Novod, N. Petrillo, D. Roazen, V. Ruano-Rubio, A. Saltzman, M. Schleicher, J. Soto, K. Tibbetts, C. Tolonen,

G. Wade, M. E. Talkowski, Genome Aggregation Database Consortium, B. M. Neale, M. J. Daly and D. G. MacArthur, The mutational constraint spectrum quantified from variation in 141,456 humans, *Nature* **581** (2020) 434–443; https://doi.org/10.1038/s41586-020-2308-7

117. NIH – NLM – NCBI, *dbSNP*; https://www.ncbi.nlm.nih.gov/snp/; last access date July 23, 2024.

118. E.-M. S. Grimm-Günter, M. Milbrandt, B. Merkl, M. Paulsson and M. Plomann, PACSIN proteins bind tubulin and promote microtubule assembly, *Exp. Cell. Res.* **314**(10) (2008) 1991–2003; https://doi.org/10.1016/j.yexcr.2008.03.015

119. H. Wint, J. Li, T. Abe, H. Yamada, T. Higaki, Y. Nasu, M. Watanabe, K. Takei and T. Takeda, Pacsin 2-dependent N-cadherin internalization regulates the migration behaviour of malignant cancer cells, *J. Cell Sci.* **136**(10) (2023) jcs260827; https://doi.org/10.1242/jcs.260827

120. G. Stocco, W. Yang, K. R. Crews, W. E. Thierfelder, G. Decorti, M. Londero, R. Franca, M. Rabusin, M. G. Valsecchi, D. Pei, C. Cheng, S. W. Paugh, L. B. Ramsey, B. Diouf, J. R. McCorkle, T. S. Jones, C.-H. Pui, M. V. Relling and W. E. Evans, PACSIN2 polymorphism influences TPMT activity and mercaptopurine-related gastrointestinal toxicity, *Hum. Mol. Genet.* **21**(21) (2012) 4793–4804; https://doi.org/10.1093/hmg/dds302

121. R. Franca, G. Stocco, D. Favretto, N. Giurici, I. del Rizzo, F. Locatelli, L. Vinti, A. Biondi, A. Colombini, F. Fagioli, E. Barisone, M. Pelin, S. Martellossi, A. Ventura, G. Decorti and M. Rabusin, PACSIN2 rs2413739 influence on thiopurine pharmacokinetics: validation studies in pediatric patients, *Pharmacogenomics J.* **20** (2020) 415–425; https://doi.org/10.1038/s41397-019-0130-0

122. A. Smid, N. Karas-Kuzelicki, J. Jazbec and I. Mlinaric-Rascan, *PACSIN2* polymorphism is associated with thiopurine-induced hematological toxicity in children with acute lymphoblastic leukaemia undergoing maintenance therapy, *Sci. Rep.* **6** (2016) Article ID 30244 (10 pages); https://doi.org/10.1038/srep30244

123. T. Moriyama, S. Liu, J. Li, J. Meyer, X. Zhao, W. Yang, Y. Shao, R. Heath, A. Hnízda, W. L. Carroll and J. J. Yang, Mechanisms of NT5C2-mediated thiopurine resistance in acute lymphoblastic leukemia, *Mol. Cancer Ther.* **18**(10) (2019) 1887–1895; https://doi.org/10.1158/1535-7163.MCT-18-1112

124. C. Jiang, W. Yang, T. Moriyama, C. Liu, C. Smith, W. Yang, M. Qian, Z. Li, M. Tulstrup, K. Schmiegelow, K. R. Crews, H. Zhang, C.-H. Pui, W. Evans, M. Relling, S. Bhatia and J. J. Yang, Effects of NT5C2 germline variants on 6-mecaptopurine metabolism in children with acute lymphoblastic leukemia, *Clin. Pharmacol. Ther.* **109**(6) (2021) 1538–1545; https://doi.org/10.1002/cpt.2095

125. M. Tulstrup, M. Grosjean, S. N. Nielsen, K. Grell, B. O. Wolthers, P. S. Wegener, O. G. Jonsson, B. Lund, A. Harila-Saari, J. Abrahamsson, G. Vaitkeviciene, K. Pruunsild, N. Toft, M. Holm, E. Hulegårdh, S. Liestøl, L. Griskevicius, M. Punab, J. Wang, W. L. Carroll, Z. Zhang, M. D. Dalgaard, R. Gupta, J. Nersting and K. Schmiegelow, *NT5C2* germline variants alter thiopurine metabolism and are associated with acquired *NT5C2* relapse mutations in childhood acute lymphoblastic leukaemia, *Leukemia* **32** (2018) 2527–2535; https://doi.org/10.1038/s41375-018-0245-3

126. T. Moriyama, J. Meyer, S. Liu, X. Zhao, Z. Ying, W. L. Carroll and J. J. Yang, NT5C2 as a major contributor to thiopurine resistance at ALL relapse via multiple mechanisms, *Blood* **126**(23) (2015) Article ID 446; https://doi.org/10.1182/blood.V126.23.446.446

127. S. Somazu, Y. Tanaka, M. Tamai, A. Watanabe, K. Kagami, M. Abe, D. Harama, T. Shinohara, K. Akahane, K. Goi, K. Sugita, T. Moriyama, J. Yang, H. Goto, M. Minegishi, S. Iwamoto, J. Takita and T. Inukai, NUDT15 polymorphism and NT5C2 and PRPS1 mutations influence thiopurine sensitivity in acute lymphoblastic leukaemia cells, *J. Cell. Mol. Med.* **25**(22) (2021) 10521–10533; https://doi.org/10.1111/jcmm.16981

128. Y. Lee, E. J. Jang, H.-Y. Yoon, J. Yee and H.-S. Gwak, Effect of ITPA polymorphism on adverse drug reactions of 6-mercaptopurine in pediatric patients with acute lymphoblastic leukemia: a systematic review and meta-analysis, *Pharmaceuticals* (Basel) **15**(4) (2022) Article ID 416 (12 pages); https://doi.org/10.3390/ph15040416

129. J. M. Van Dieren, B. E. Hansen, E. J. Kuipers, E. E. S. Nieuwenhuis and C. J. Van der Woude, Meta-analysis: Inosine triphosphate pyrophosphatase polymorphisms and thiopurine toxicity in the treatment of inflammatory bowel disease, *Aliment. Pharmacol. Ther.* **26**(5) (2007) 643–652; https://doi.org/10.1111/j.1365-2036.2007.03412.x

130. R. Steponaitiene, J. Kupcinskas, S. Survilaite, G. Varkalaite, L. Jonaitis, G. Kiudelis, G. Denapiene, J. Valantinas, J. Skieceviciene and L. Kupcinskas, *TPMT* and *ITPA* genetic variants in Lithuanian inflammatory bowel disease patients: Prevalence and azathioprine-related side effects, *Adv. Med. Sci.* **61**(1) (2016) 135–140; https://doi.org/10.1016/j.advms.2015.09.008

131. A. Jena, N. Grover, P. Bhatia, M. Singh, D. Lad, K. K. Prasad, H. Singh, U. Dutta and V. Sharma, ITPA polymorphisms do not predict additional risk beyond TPMT and NUDT15 for thiopurine-induced cytopenia in inflammatory bowel disease ITPA polymorphisms do not predict additional risk beyond TPMT and NUDT15 for thiopurine-induced cytopenia in inflammatory bowel disease [Los polimorfismos de ITPA no predicen un riesgo adicional más allá de TPMT y NUDT15 para citopenia inducida por tiopurina en la enfermedad inflamatoria intestinal], *Rev. Gastroenterol. Mex.* (Engl. Ed.) **89**(1) (2024) 25–30; https://doi.org/10.1016/j.rgmxen.2021.11.017

132. Y. S. Jung, J. H. Cheon, J. J. Park, C. M. Moon, E. S. Kim, J. H. Lee, S. W. Kim, J. H. Kim, S. P. Hong, T. I. Kim and W. H. Kim, Correlation of genotypes for thiopurine methyltransferase and inosine triphosphate pyrophosphatase with long-term clinical outcomes in Korean patients with inflammatory bowel diseases during treatment with thiopurine drugs, *J. Hum. Genet.* **55** (2010) 121–123; https://doi.org/10.1038/jhg.2009.125

133. Q. Miao, L. Yan, Y. Zhou, Y. Li, Y. Zou, L. Wang, Y. Bai and J. Zhang, Association of genetic variants in *TPMT*, *ITPA*, and *NUDT15* with azathioprine-induced myelosuppression in southwest china patients with autoimmune hepatitis, *Sci. Rep.* **11** (2021) Article ID 7984 (8 pages); https://doi.org/10.1038/s41598-021-87095-0

134. T. Adam de Beaumais, M. Fakhoury, Y. Medard, S. Azougagh, D. Zhang, K. Yakouben and E. Jacqz-Aigrain, Determinants of mercaptopurine toxicity in paediatric acute lymphoblastic leukemia maintenance therapy, *Br. J. Clin. Pharmacol.* **71**(4) (2011) 575–584; https://doi.org/10.1111/j.1365-2125.2010.03867.x

135. R. Choi, M.-N. Lee, K. Kim, S.-Y. Baek, T. J. Kim, S. N. Hong, Y.-H. Kim and S.-Y. Lee, Effects of various genetic polymorphisms on thiopurine treatment-associated outcomes for Korean patients with Crohn's disease, *Br. J. Clin. Pharmacol.* **86**(11) (2020) 2302–2313; https://doi.org/10.1111/bcp.14339

136. T. Gerbek, M. Ebbesen, J. Nersting, T. L. Frandsen, M. L. Appell and K. Schmiegelow, Role of TPMT and ITPA variants in mercaptopurine disposition, *Cancer Chemother. Pharmacol.* **81** (2018) 579–586; https://doi.org/10.1007/s00280-018-3525-8

137. B. Moradveisi, S. Muwakkit, F. Zamani, E. Ghaderi, E. Mohammadi and N. K. Zgheib, *ITPA*, *TPMT*, and *NUDT15* genetic polymorphisms predict 6-mercaptopurine toxicity in Middle Eastern children with acute lymphoblastic leukemia, *Front. Pharmacol.* **10** (2019) Article ID 916 (8 pages); https://doi.org/10.3389/fphar.2019.00916

138. B. Boonyawat, C. Monsereenusorn, A. Photia, N. Lertvivatpong, V. Kaewchaivijit, P. Jindatanmanusan and P. Rujkijyanont, ITPA:c.94C>A and NUDT15:c.415C>T polymorphisms and their relation to mercaptopurine-related myelotoxicity in childhood leukemia in Thailand, *Appl. Clin. Genet.* **14** (2021) 341–351; https://doi.org/10.2147/TACG.S318912

139. A. Smid, N. Karas-Kuzelicki, M. Milek, J. Jazbec and I. Mlinaric-Rascan, Association of ITPA genotype with event-free survival and relapse rates in children with acute lymphoblastic leukemia undergoing maintenance therapy, *PLoS ONE* **9**(10) (2014) Article ID e109551 (10 pages); https://doi.org/10.1371/journal.pone.0109551

140. J. Ereño-Orbea, T. Majtan, I. Oyenarte, J. P. Kraus and L. A. Martínez-Cruz, Structural insight into the molecular mechanism of allosteric activation of human cystathionine β-synthase by *S*-adeno-

sylmethionine, *Proc. Natl. Acad. Sci. USA* **111**(37) (2014) E3845-E3852; https://doi.org/10.1073/pnas.1414545111

141. T. H. Scheuermann, C. Keeler and M. E. Hodsdon, Consequences of binding an S-adenosylmethionine analogue on the structure and dynamics of the thiopurine methyltransferase protein backbone, *Biochemistry* **43**(38) (2004) 12198–12209; https://doi.org/10.1021/bi0492556

142. M. Milek, N. Karas Kuzelicki, A. Smid and I. Mlinaric-Rascan, S-adenosylmethionine regulates thiopurine methyltransferase activity and decreases 6-mercaptopurine cytotoxicity in MOLT lymphoblasts, *Biochem. Pharmacol.* **77**(12) (2009) 1845–1853; https://doi.org/10.1016/j.bcp.2009.03.006

143. N. Karas-Kuzelicki, M. Milek and I. Mlinaric-Rascan, MTHFR and TYMS genotypes influence TPMT activity and its differential modulation in males and females, *Clin. Biochem.* **43**(1–2) (2010) 37–42; https://doi.org/10.1016/j.clinbiochem.2009.09.003

144. M.-N. Lee, B. Kang, S. Y. Choi, M. J. Kim, S. Y. Woo, J.-W. Kim, Y. H. Choe and S.-Y. Lee, Impact of genetic polymorphisms on 6-thioguanine nucleotide levels and toxicity in pediatric patients with IBD treated with azathioprine, *Inflamm. Bowel Dis.* **21**(12) (2015) 2897–2908; https://doi.org/10.1097/MIB.0000000000000570

145. NIH – NLM - NCBI, Genetic Testing Registry (GTR), *Clinical and Research Tests for NUDT15*; https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=NUDT15; last access date April 17, 2024.

146. R. J. Marrero, E. J. Cicali, M. J. Arwood, E. Eddy, D. DeRemer, B. H. Ramnaraign, K. C. Daily, D. Jones Jr, K. J. Cook, L. H. Cavallari, K. Wiisanen Weitzel, T. Langaee, K. J. Newsom, P. Starostik, M. J. Clare-Salzer, J. A. Johnson, T. J. George and R. M. Cooper-DeHoff, how to transition from single-gene pharmacogenetic testing to preemptive panel-based testing: A tutorial, *Clin. Pharmacol. Ther.* **108**(3) (2020) 557–565; https://doi.org/10.1002/cpt.1912

147. J. Hayward, J. McDermott, N. Qureshi and W. Newman, Pharmacogenomic testing to support prescribing in primary care: a structured review of implementation models, *Pharmacogenomics* **22**(12) (2021) 761–776; https://doi.org/10.2217/pgs-2021-0032

148. C. H. van der Wouden, E. Paasman, M. Teichert, M. R. Crone, H.-J. Guchelaar and J. J. Swen, Assessing the Implementation of pharmacogenomic panel-testing in primary care in the Netherlands utilizing a theoretical framework, *J. Clin. Med.* **9**(3) (2020) Article ID 814 (19 pages); https://doi.org/10.3390/jcm9030814

149. M. B. Rosenman, B. Decker, K. D. Levy, A. M. Holmes, V. M. Pratt and M. T. Eadon, Lessons learned when introducing pharmacogenomic panel testing into clinical practice, *Value Health* **20**(1) (2017) 54–59; https://doi.org/10.1016/j.jval.2016.08.727

150. C. H. van der Wouden, H.-J. Guchelaar and J. J. Swen, Precision medicine using pharmacogenomic panel-testing: current status and future perspectives, *Adv. Mol. Pathol.* **3** (2020) 131–142; https://doi.org/10.1016/j.yamp.2020.07.012

151. H. Xin, C. Fischer, M. Schwab and U. Klotz, Effects of aminosalicylates on thiopurine S-methyltransferase activity: an ex vivo study in patients with inflammatory bowel disease, *Aliment. Pharmacol. Ther.* **21**(9) (2005) 1105–1109; https://doi.org/10.1111/j.1365-2036.2005.02460.x

152. R. A. Lysaa, T. Giverhaug, H. L. Wold and J. Aarbakke, Inhibition of human thiopurine methyltransferase by furosemide, bendroflumethiazide and trichlormethiazide, *Eur. J. Clin. Pharmacol.* **49** (1996) 393–396; https://doi.org/10.1007/s002280050038

153. K. Oselin and K. Anier, Inhibition of human thiopurine S-methyltransferase by various nonsteroidal anti-inflammatory drugs in vitro: a mechanism for possible drug interactions, *Drug Metab. Dispos.* **35**(9) (2007) 1452–1454; https://doi.org/10.1124/dmd.107.016287

154. J. P. A. Houwen, A. C. G. Egberts, A. de Boer, E. M. van Maarseveen, R. H. J. Houwen and A. Lalmohamed, Influence of allopurinol on thiopurine associated toxicity: A retrospective population-based cohort study, *Br. J. Clin. Pharmacol.* **87**(5) (2021) 2333–2340; https://doi.org/10.1111/bcp.14625

155. J. Brackett, E. S. Schafer, D. H. Leung and M. B. Bernhardt, Use of allopurinol in children with acute lymphoblastic leukemia to reduce skewed thiopurine metabolism, *Pediatr. Blood Cancer* **61**(6) (2014) 1114–1117; https://doi.org/10.1002/pbc.24913

156. S. F. Chavoushi, B. Jharap, P. Friedrich, K. Smid, G. J. Peters and M. Malingré, Thiopurines with low-dose allopurinol (ThiLDA) – a prospective clinical one-way crossover trial, *Eur. J. Clin. Pharmacol.* **75** (2019) 1669–1674; https://doi.org/10.1007/s00228-019-02760-8

157. G. Nakafero, T. Card, M. J. Grainge, H. C. Williams, M. W. Taal, G. P. Aithal, C. P. Fox, C. D. Mallen, D. A. van der Windt, M. D. Stevenson, R. D. Riley and A. Abhishek, Risk-stratified monitoring for thiopurine toxicity in immune-mediated inflammatory diseases: prognostic model development, validation, and, health economic evaluation, *eClin. Med.* **64** (2023) Article ID 102213 (13 pages); https://doi.org/10.1016/j.eclinm.2023.102213

158. G. Milosevic, N. Kotur, N. Krstovski, J. Lazic, B. Zukic, B. Stankovic, D. Janic, T. Katsila, G. P. Patrinos, S. Pavlovic and L. Dokmanovic, Variants in TPMT, ITPA, ABCC4 and ABCB1 genes as predictors of 6-mercaptopurine induced toxicity in children with acute lymphoblastic leukemia, *J. Med. Biochem.* **37**(3) (2018) 320–327; https://doi.org/10.1515/jomb-2017-0060

159. N. H. Nguyen, D. Picetti, P. S. Dulai, V. Jairath, W. J. Sandborn, L. Ohno-Machado, P. L. Chen and S. Singh, Machine learning-based prediction models for diagnosis and prognosis in inflammatory bowel diseases: A systematic review, *J. Crohns Colitis* **16**(3) (2022) 398–413; https://doi.org/10.1093/ecco-jcc/jjab155

160. L. Arosa, M. Camba-Gómez, O. Golubnitschaja and J. Conde-Aranda, Predictive, preventive and personalised approach as a conceptual and technological innovation in primary and secondary care of inflammatory bowel disease benefiting affected individuals and populations, *EPMA J.* **15** (2024) 111–123; https://doi.org/10.1007/s13167-024-00351-x