Relevance of pharmacogenetic aspects of mercaptopurine metabolism in the treatment of interstitial lung disease

Jaap A. Bakker[a,b], Marjolein Drent[c] and Jörgen Bierau[a]

Purpose of review

Mercaptopurine therapy is increasingly important as immunosuppressive therapy in interstitial lung disease. We focus on human mercaptopurine metabolism and the defects in this metabolism causing adverse drug reactions.

Recent findings

Defects in mercaptopurine metabolizing enzymes like thiopurine methyltransferase and inosine triphosphate pyrophosphohydrolase lead to severe adverse drug reactions, sometimes with fatal outcome. Other enzymes, still not thoroughly investigated, can give rise to toxic effects or decreased efficacy in mercaptopurine therapy when the activity of these enzymes is altered.

Summary

Pharmacogenetic screening of potential patients for mercaptopurine therapy is important to avoid adverse drug reactions caused by inherited enzyme deficiencies in these metabolic pathways. Pretreatment screening for deficiencies of mercaptopurine metabolizing enzymes will significantly reduce the number of patients with an adverse drug reaction and concomitantly associated healthcare costs.

Keywords

adverse drug events, inosine triphosphate pyrophosphohydrolase, mercaptopurine metabolism, pharmacogenetic screening, thiopurine methyltransferase

Curr Opin Pulm Med 13:458–463. © 2007 Lippincott Williams & Wilkins.

[a]Department of Clinical Genetics, [b]Department of Clinical Chemistry and [c]Department of Respiratory Medicine, University Hospital Maastricht, Maastricht, The Netherlands

Correspondence to Jaap A. Bakker, MSc, Laboratory for Inherited Metabolic Diseases, Department of Clinical Genetics, University Hospital Maastricht, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands
Tel: +31 43 387 7835; fax: +31 43 387 7901;
e-mail: jaap.bakker@gen.unimaas.nl

Current Opinion in Pulmonary Medicine 2007, 13:458–463

Abbreviations

5′-NT	purine-5′-nucleotidase
6-MMP	6-methylmercaptopurine
6-MP	6-mercaptopurine
6-TG	6-thioguanine
6-TGN	6-thioguanine nucleotide
6-TIMP	6-thioinosine monophosphate
6-TITP	6-thioinosine triphosphate
6-TGMP	6-thioguanine monophosphate
ADR	adverse drug reaction
GMPS	guanosine-monophosphate synthetase
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
ILD	interstitial lung disease
IMPDH	inosine monophosphate dehydrogenase
ITPase	inosine triphosphate pyrophosphohydrolase
SAM	S-adenosyl methionine
TPMT	mercaptopurine methyltransferase

1070-5287

Introduction

Interstitial lung disease (ILD), including idiopathic interstitial pneumonias, is a heterogeneous group of diffuse parenchymal lung diseases [1,2••]. The clinical outcome of ILD can be favorable, however in a substantial percentage of patients the course is progressive. Examples are patients suffering from idiopathic pulmonary fibrosis and severe sarcoidosis. In a substantial number of those cases immunosuppressive drugs, like mercaptopurines, are potential candidates in the treatment regimens [3–8]. In 3–5% of the patients these drugs give rise to serious adverse drug reactions (ADRs) like myelosuppression, elevated liver functions, pancreatitis and flu-like symptoms [9]. In immune-compromised patients this will lead to hospitalization or at least intensified medical surveillance.

Most of these ADRs are due to a reduced or absent activity of drug metabolizing enzyme systems. These defects may be either genetically determined or acquired [10]. The study of the interaction between genetic make up and therapeutic compounds is termed pharmacogenetics.

Drug metabolism is divided in two phases – phase I and phase II. Phase I reactions include oxidation, reduction and hydrolysis of the drug. The cytochrome P-450 group of enzymes mediates the majority of these reactions. Phase II drug metabolism includes reactions in which the drug is conjugated, that is acetylated, glucuronidated or methylated [11–13].

Mercaptopurines are a class of immunosuppressive antimetabolites used for the treatment of a wide array of

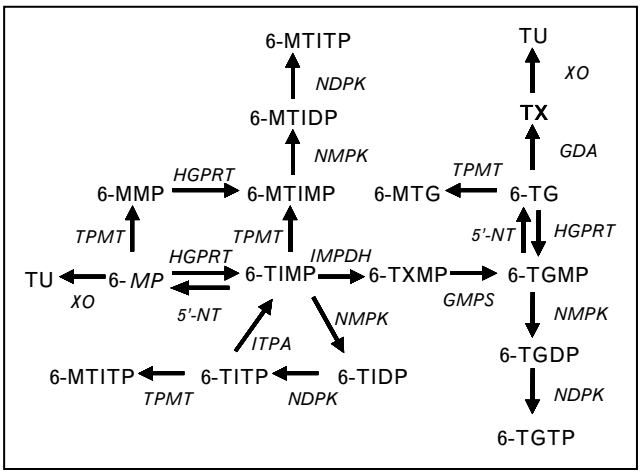
diseases, including ILD. Due to their extensive use, ADRs in mercaptopurine-based therapeutic regimens occur frequently, potentially having fatal consequences. In this paper we will address ADRs caused by mercaptopurine therapy in relation to defects in purine metabolism.

Mercaptopurine metabolism

The mercaptopurine-based drugs, azathioprine, 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) are used as immunosuppressants in the treatment of autoimmune disorders, inflammatory bowel disease, debilitating skin diseases and various inflammatory eye conditions and as antiproliferative drugs in childhood acute lymphoblastic leukemia [14••,15•,16,17•,18•].

Azathioprine is a pro-drug of 6-MP, which is activated by glutathione-S-transferase-dependent enzymatic cleavage, releasing the methyl-nitro-thioimidazole moiety from the azathioprine molecule, generating 6-MP (see Fig. 1). Approximately 1% of the 6-MP thus generated is activated by hypoxanthine-guanine phosphoribosyl transferase (HGPRT), a salvage enzyme in purine metabolism, producing 6-thioinosine monophosphate (6-TIMP). Eighty-four per cent of the activated 6-MP is subjected to oxidation by xanthine oxidase to 6-thiouric acid. Aldehyde oxidase is also capable of oxidizing 6-MP: it catalyzes the conversion of 6-MP and 6-TG to their 8-hydroxy analogues. The importance of this deactivation pathway is less clear [19,20].

Figure 1 Mercaptopurine metabolism in humans



GDA, guanine deaminase; GMPS, guanosine monophosphate synthase; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; IMPDH, inosine monophosphate dehydrogenase; ITPA, inosine triphosphate pyrophosphohydrolase; 6-MP, 6-mercaptopurine; 6-MMP, 6-methylmercaptopurine; 6-MTIM(D/T)P, 6-methylthioinosine mono(di/tri)phosphate; 6-MTG, 6-methylthioguanine; NM(D)PK, nucleotide mono(di)phosphate kinase; 5′-NT, 5′-nucleotidase; 6-TG, 6-thioguanine; 6-TGM(D/T)P, 6-thioguanine mono(di/tri)phosphate; TPMT, thiopurine-S-methyltransferase; TU, thiouric acid; TX, thioxanthine; 6-TXMP, 6-thioxanthosine monophosphate; XO, xanthine oxidase.

Another important deactivation route, handling 15% of the generated 6-MP, is methylation catalyzed by mercaptopurine methyltransferase (TPMT). Deactivation of 6-MP by TPMT yields 6-methylmercaptopurine (6-MMP) [21,22].

After activation of mercaptopurine to 6-TIMP, this metabolite is further metabolized to 6-thioguanine monophosphate (6-TGMP) by successively inosine-monophosphate dehydrogenase (IMPDH) and guanosine-monophosphate synthetase (GMPS). 6-TGMP is the precursor of the cytotoxic 6-thioguanine (deoxy) ribonucleotide 5′-triphosphates, which are incorporated into DNA and RNA, respectively.

As shown in Fig. 1, 6-TIMP is also deactivated by TPMT, resulting in the formation of 6-Me-TIMP.

6-TG is activated in a similar fashion as 6-MP. The first activation step is catalyzed by HGPRT, yielding 6-TGMP. Further metabolization is catalyzed by nucleotide kinases, resulting in the formation of the above-mentioned cytotoxic (deoxy)ribonucleotide 5′-triphosphates of 6-thioguanine. 6-TG is deactivated either by methylation by TPMT or by degradation through guanine deaminase and xanthine oxidase to thiouric acid.

Another key enzyme in mercaptopurine metabolism is inosine triphosphate pyrophosphohydrolase (ITPase). This enzyme is responsible for the pyrophosphohydrolysis of 6-thioinosine triphosphate (6-TITP), one of the metabolites of 6-TIMP. Deficiency or a decreased activity of this enzyme results in a surplus of (thio)ionosine triphosphates, which disturbs the equilibrium in the cellular (deoxy) nucleotide triphosphate pool: for example, ITP can compete with GTP in GTP-driven reactions [23].

Another pathway for detoxification of mercaptopurine is degradation of nucleotides to nucleosides catalyzed by purine-5′-nucleotidase (5′-NT). Both 6-TIMP and 6-TGMP are substrates for 5′-NT, although at higher concentrations of these thionucleotides substrate inhibition has been observed [24].

Pharmacogenetic defects responsible for mercaptopurine toxicity

As can be seen in Fig. 1, mercaptopurine metabolism is an intricate process involving a large number of enzymes. It is not hard to imagine that a decreased activity of one of the enzymes involved in this process gives rise to an accumulation of metabolites. Depending on which enzyme is deficient, metabolites can accumulate in specific tissues and reach concentration levels at which they become cytotoxic, thus giving rise to ADR. The characteristics are summarized in Table 1.

Table 1 Characteristics of pharmacogenetic defects of mercaptopurine metabolism

Enzyme deficit	Metabolic consequence	Clinical presentation
Hypoxanthine-guanine phosphoribosyltransferase	Decreased efficacy of thiopurinenucleotide formation	Nonresponsiveness
Xanthine oxidase	Increased formation of thioguaninenucleotides	Leuco/pancytopenia
Thiopurinemethyltransferase	Increased formation of thioguaninenucleotides	Leuco/pancytopenia
Inosine-triphosphate pyrophosphohydrolase	Putative accumulation of 6-methylthio-ITP	Flu-like syndromes, pancreatitis, leuco/pancytopenia rash
5′nucleotidase	Decreased efficacy of thioguaninenucleotide formation	Leuco/pancytopenia
Inosine monophosphate dehydrogenase	Decreased efficacy of thioguaninenucleotide formation	Unknown
Guanosine monophosphate synthetase	Decreased efficacy of thioguaninenucleotide formation	Unknown

Although nonresponsiveness to mercaptopurine therapy is not per definition an ADR, it is worthwhile mentioning this phenomenon in the current context.

Nonresponsiveness in mercaptopurine therapy in the case of azathioprine can be due to a diminished activity of glutathione-S-transferase or a lack of available glutathione. Prolonged treatment with azathioprine/6-MP can give rise to a depletion of available glutathione [5,25••].

Another frequently noted reaction to azathioprine therapy is the occurrence of a rash. This is assigned to an allergic reaction to the release of the imidazole moiety of azathioprine [26].

Recently, nonresponsiveness to mercaptopurine therapy in human leukemia cell lines was associated with impaired transport of mercaptopurines into the cell [27••].

As was pointed out before, activation of 6-MP to 6-TIMP is mediated by HGPRT. A complete deficiency of this enzyme leads to the clinically distinct phenotype of Lesch Nyhan syndrome; the partial deficiency mainly occurs in females and presents with a much milder phenotype. When 6-MP is given to patients with partial hypoxanthine phosphoribosyltransferase (HPRT) deficiency a diminished response on 6-MP therapy, as measured by 6-thioguanine nucleotide (6-TGN) formation, is noted. There are high concentrations of 6-MMP metabolites relative to the concentration of 6-TGN in peripheral red blood cells. Substituting 6-TG for 6-MP is of no use, because 6-TG activation also depends on HPRT [28].

A diminished in-vivo activity of xanthine oxidase may have serious consequences in mercaptopurine therapy. About 85% of the bio-available 6-MP is deactivated by xanthine oxidase to thiouric acid in the liver and the intestine [29]. After deamination by guanidine deaminase, 6-TG is deactivated by the same pathway. Theoretically a lowered xanthine oxidase activity will result in an increased availability of 6-MP for either methylation by TPMT or further activation to 6-TIMP by HPRT. The result is an increased concentration of 6-MMP relative to 6-TGN concentrations. As far as we know, patients with an increased 6-MMP and relatively normal amount of 6-TG are not routinely checked for xanthine oxidase activity [21]. Studies using a combination therapy of 6-MP and allopurinol, a xanthine oxidase inhibitor, in patients not responding to 6-MP/azathioprine therapy showed an increase in 6-TGN availability when compared with azathioprine therapy only. The doses of azathioprine in the combined therapy were lowered in order to avoid toxic effects on the bone marrow of an increased supply of 6-MP. This finding suggests a key role for xanthine oxidase in mercaptopurine therapy, in which tissue distribution of xanthine oxidase may be of importance in understanding this role [21,30]. The antifolate drug methotrexate also has an inhibitory effect on xanthine oxidase and care has to be taken when methotrexate is combined with 6-MP/azathioprine therapy [29].

The most extensively studied enzyme in the mercaptopurine utilization pathway is TPMT [31••]. As is described by Gunnarsdottir *et al.* [32], TPMT is active at nearly all steps of the mercaptopurine pathway. The importance of TPMT is underlined in the effects that appear when the activity of the enzyme is altered, both decreased or increased [33•].

TPMT activity in the normal population has a trimodal distribution: 0.3% low activity, 11% intermediate activity and 89% normal to high activity [34]. A high activity of TPMT results in the formation of relatively high intracellular concentrations of 6-MMP and subsequently a decreased concentration of 6-TGN. In these patients the efficacy of azathioprine or 6-MP is less than in patients with a normal or low TPMT activity as the 6-TGNs are the therapeutically active metabolites [35]. High levels of 6-MMP are associated with hepatotoxicity (elevated transaminases) [36,37•]. The methylation product of TIMP, 6-MTIMP, inhibits the purine de-novo synthesis. The consequence of this inhibition is a depletion of the available (deoxy)nucleotides necessary for DNA and RNA synthesis [38].

The methyl group necessary for the methylation of 6-MP and its nucleotides is donated by S-adenosyl methionine (SAM). An enhanced methylation rate may deplete SAM, possibly interfering with other metabolic processes requiring SAM.

A decreased or absent TPMT activity results in increased synthesis of 6-TG metabolites from 6-MP. Especially in tissues without xanthine oxidase activity, like the bone marrow, 6-TGN will accumulate in high levels causing serious life-threatening myelosuppression [39•,40•]. For obvious reasons the dosage of 6-MP has to be reduced in order to avoid the ADR.

Recently the role of folic acid and the enzyme methylene-tetrahydrofolate reductase (MTHFR), which catalyses the (re)methylation of homocysteine to methionine in mercaptopurine metabolism, has been studied. Breen *et al.* [22] studied the possible role of mutations in the *MTHFR* gene. There appeared, however, to be no correlation between polymorphisms in the gene encoding MTHR and azathioprine toxicity.

Due to the crucial role that TPMT has in mercaptopurine metabolism, measurement of erythrocyte TPMT activity is recommended before starting mercaptopurine-based treatment. In case a decreased TPMT activity is found, the therapeutic regimen can be adjusted. In daily clinical practice, TPMT phenotyping, that is measurement of TPMT activity, is preferred to genotyping. The results of TPMT phenotyping are usually available within 5 working days and exclude the risk of overlooking/missing novel mutations. Genotyping is used to establish the genetic base of a decreased activity of the enzyme. Ninety-five per cent of the reduced TPMT activities is accounted for by three common polymorphisms, that is *TMPT∗2*, *∗3A* and *∗3C* [41].

Another enzyme involved in mercaptopurine metabolism having pharmacogenetic consequences is ITPase [23,42]. As shown in Fig. 1, ITPase catalyses the pyrophosphohydrolysis of ITP to IMP. In mercaptopurine metabolism TITP is generated from TIMP, which is cycled back to TIMP by ITPase. TITP is also methylated by TPMT to 6-MTITP. The accumulating metabolites, TITP and 6-MTITP, are so-called 'rogue' nucleotides and may interfere with other nucleotides in RNA incorporation.

As with TPMT, the activity of ITPase is trimodally distributed. Lower activities are due to homozygosity or compound heterozygosity for one of the inactivating mutations in the *ITPA* gene. Heterozygous individuals for the $94C \rightarrow A$ polymorphism have low intermediate activities; patients with one $IVS2 + 21A \rightarrow C$ polymorphism have high intermediate or low normal activities of ITPase in peripheral red blood cells [43,44•,45•]. There are conflicting reports in the literature on the toxicity of mercaptopurines in heterozygotes and homozygous individuals [46–48]. It is recommended to adapt the therapeutic regimen by changing from 6-MP to 6-TG (Lanvis) in the case of decreased ITPase activity to avoid ADRs [39•,49].

Deficiency of 5′-NT leads to increased concentrations of 6-TGMP and 6-TIMP, the latter being methylated by TPMT to 6-MTIMP. In terms of ADRs, the consequences are inhibition of the purine de-novo synthesis by 6-MTIMP and higher cytotoxicity through accumulation of 6-TGMP [50,51]. Although nowadays the focus is on TMPT and ITPase we consider this enzyme may be of equal importance in research on ADRs in mercaptopurine treatment.

Further activation of the mercaptopurines is catalyzed by IMPDH and GMPS. The role of IMPDH in mercaptopurine activation is still unclear. Theoretically one can presume ADRs in case of a reduced activity of IMPDH, which is the rate-limiting step in guanine nucleotide biosynthesis [52]. Investigations of IMPDH are mostly linked to the use of mycophenolic acid (MPA). MPA is an inhibitor of mammalian IMPDH and therefore can suppress de-novo GTP synthesis [53]. Azathioprine resistance in one patient was associated with a mutation in the P3 promotor region of *IMPDH1* [54•].

Another enzyme involved in the GMP biosynthesis is GMPS. There is a suggestion in the literature about the possibility of using this enzyme in immunomodulating processes [55].

Conclusion

The metabolism of mercaptopurines is an intricate network, requiring many enzymes. Defects in any of these enzymes will cause an alteration in mercaptopurine metabolism, some giving rise to the accumulation of highly toxic metabolites causing ADRs. In order to avoid ADRs it is strongly recommended that all patients, including patients suffering from ILD who are due to be treated with mercaptopurine (analogs) for disturbances in the activation/deactivation pathway of mercaptopurines, are tested for TPMT and ITPase activity before initiating therapy [44•,56].

With respect to the other enzymes involved in mercaptopurine metabolism, 5-NT, HGPRT and IMPDH are candidate enzymes for further investigation. Measurement of the enzyme activity is the first step in the process; mutation analysis of the gene is the second. The additional costs will be limited by this multi-step approach. During mercaptopurine therapy the patient must be monitored on a regular basis, both by measuring erythrocyte thionucleotide concentrations and by blood counts.

This approach is of high clinical relevance as it aims to reduce healthcare costs and to improve patients' quality of life [57••,58••,59].

Including pharmacogenetics in therapeutic drug monitoring will lead to a more individualized pharmacotherapy

[60]. Therefore, there is a need for implementation of this knowledge in daily clinical practice to ensure the benefits will be achieved [61].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 469).

1 American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 2002; 165:277–304.

2 •• Martinez FJ. Idiopathic interstitial pneumonias: usual interstitial pneumonia versus nonspecific interstitial pneumonia. Proc Am Thorac Soc 2006; 3:81–95.
Excellent overview of the diagnosis and treatment options for idiopathic interstitial pneumonia.

3 Davies HR, Richeldi L, Walters EH. Immunomodulatory agents for idiopathic pulmonary fibrosis. Cochrane Database Syst Rev 2003:CD003134.

4 Hull JH. Acetylcysteine in pulmonary fibrosis. N Engl J Med 2006; 354:1089–1091.

5 Demedts M, Behr J, Buhl R, *et al.* High-dose acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med 2005; 353:2229–2242.

6 Shah NR, Noble P, Jackson RM, *et al.* A critical assessment of treatment options for idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2005; 22:167–174.

7 Raghu G, Depaso WJ, Cain K, *et al.* Azathioprine combined with prednisone in the treatment of idiopathic pulmonary fibrosis: a prospective double-blind, randomized, placebo-controlled clinical trial. Am Rev Respir Dis 1991; 144: 291–296.

8 Drent M, du Bois RM, Poletti V. Recent advances in the diagnosis and management of nonspecific interstitial pneumonia. Curr Opin Pulm Med 2003; 9:411–417.

9 Sanderson J, Ansari A, Marinaki T, Duley J. Thiopurine methyltransferase: should it be measured before commencing thiopurine drug therapy? Ann Clin Biochem 2004; 41:294–302.

10 Ferraccioli G, De Santis M, Tolusso B. Pharmacogenetics/pharmacogenomics and antirheumatic drugs in rheumatology. Pharmacogenomics 2004; 5:1107–1116.

11 Weinshilboum R. Inheritance and drug response. N Engl J Med 2003; 348:529–537.

12 Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999; 286:487–491.

13 Hiratsuka M, Sasaki T, Mizugaki M. Genetic testing for pharmacogenetics and its clinical application in drug therapy. Clin Chim Acta 2006; 363:177–186.

14 •• Derijks LJ, Gilissen LP, Hooymans PM, Hommes DW. Review article: thiopurines in inflammatory bowel disease. Aliment Pharmacol Ther 2006; 24:715–729.
Recent review on the use of thiopurines in inflammatory bowel disease and the possible strategies to optimize mercaptopurine therapy.

15 • Banerjee S, Bishop WP. Evolution of thiopurine use in pediatric inflammatory bowel disease in an academic center. J Pediatr Gastroenterol Nutr 2006; 43:324–330.
The focus of this paper is on the pediatric use of mercaptopurines in inflammatory bowel disease.

16 Clunie GP, Lennard L. Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine. Rheumatology (Oxford) 2004; 43:13–18.

17 • Gauba V, Saldanha M, Vize C, Saleh GM. Thiopurine methyltransferase screening before azathioprine therapy. Br J Ophthalmol 2006; 90:923–924.
Short report on the use of mercaptopurine therapy in ophthalmology.

18 • Meggitt SJ, Gray JC, Reynolds NJ. Azathioprine dosed by thiopurine methyltransferase activity for moderate-to-severe atopic eczema: a double-blind, randomised controlled trial. Lancet 2006; 367:839–846.
Well designed study on the efficacy of mercaptopurine therapy in patients with atopic eczema.

19 Coulthard SA, Hogarth LA, Little M, *et al.* The effect of thiopurine methyltransferase expression on sensitivity to thiopurine drugs. Mol Pharmacol 2002; 62:102–109.

20 Kitchen BJ, Moser A, Lowe E, *et al.* Thioguanine administered as a continuous intravenous infusion to pediatric patients is metabolized to the novel metabolite 8-hydroxy-thioguanine. J Pharmacol Exp Ther 1999; 291:870–874.

21 Duley JA, Florin TH. Thiopurine therapies: problems, complexities, and progress with monitoring thioguanine nucleotides. Ther Drug Monit 2005; 27:647–654.

22 Breen DP, Marinaki AM, Arenas M, Hayes PC. Pharmacogenetic association with adverse drug reactions to azathioprine immunosuppressive therapy following liver transplantation. Liver Transpl 2005; 11:826–833.

23 Sumi S, Marinaki AM, Arenas M, *et al.* Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. Hum Genet 2002; 111:360–367.

24 Brouwer C, Vogels-Mentink TM, Keizer-Garritsen JJ, *et al.* Role of 5′-nucleotidase in thiopurine metabolism: enzyme kinetic profile and association with thio-GMP levels in patients with acute lymphoblastic leukemia during 6-mercaptopurine treatment. Clin Chim Acta 2005; 361:95–103.

25 •• Stocco G, Martelossi S, Barabino A, *et al.* Glutathione-S-transferase genotypes and the adverse effects of azathioprine in young patients with inflammatory bowel disease. Inflamm Bowel Dis 2007; 13:57–64.
Excellent paper on the role of glutathione-S-transferase mutations and the therapeutic efficacy of mercaptopurine therapy.

26 McGovern DP, Travis SP, Duley J, *et al.* Azathioprine intolerance in patients with IBD may be imidazole-related and is independent of TPMT activity. Gastroenterology 2002; 122:838–839.

27 •• Fotoohi AK, Wrabel A, Moshfegh A, *et al.* Molecular mechanisms underlying the enhanced sensitivity of thiopurine-resistant T-lymphoblastic cell lines to methyl mercaptopurineriboside. Biochem Pharmacol 2006; 72:816–823.
This paper opens a complete new field in mercaptopurine research by describing the role of transporters in cellular transport of mercaptopurine and its metabolites.

28 Bemi V, Turchi G, Margotti E, *et al.* 6-Thioguanine resistance in a human colon carcinoma cell line with unaltered levels of hypoxanthine guanine phosphoribosyltransferase activity. Int J Cancer 1999; 82:556–561.

29 Giverhaug T, Loennechen T, Aarbakke J. The interaction of 6-mercaptopurine (6-MP) and methotrexate (MTX). Gen Pharmacol 1999; 33:341–346.

30 Chocair P, Duley J, Simmonds HA, *et al.* Low-dose allopurinol plus azathioprine/cyclosporin/prednisolone, a novel immunosuppressive regimen. Lancet 1993; 342:83–84.

31 •• Weinshilboum RM. Pharmacogenomics: catechol O-methyltransferase to thiopurine S-methyltransferase. Cell Mol Neurobiol 2006; 26:539–561.
In this outstanding paper the clinical relevance of methyltransferases in human metabolism is described in pharmacogenetic terms.

32 Gunnarsdottir S, Elfarra AA. Distinct tissue distribution of metabolites of the novel glutathione-activated thiopurine prodrugs cis-6-(2-acetylvinylthio)purine and trans-6-(2-acetylvinylthio)guanine and 6-thioguanine in the mouse. Drug Metab Dispos 2003; 31:718–726.

33 • Derijks LJ, Gilissen LP, de Boer NK, Mulder CJ. 6-Thioguanine-related hepatotoxicity in patients with inflammatory bowel disease: dose or level dependent? J Hepatol 2006; 44:821–822.
The relation between thioguanine nucleotides and hepatic toxicity is described in patients on mercaptopurine therapy.

34 Dubinsky MC, Lamothe S, Yang HY, *et al.* Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology 2000; 118:705–713.

35 Nagasubramanian R, Innocenti F, Ratain MJ. Pharmacogenetics in cancer treatment. Annu Rev Med 2003; 54:437–452.

36 Nygaard U, Toft N, Schmiegelow K. Methylated metabolites of 6-mercaptopurine are associated with hepatotoxicity. Clin Pharmacol Ther 2004; 75:274–281.

37 • Gilissen LP, Derijks LJ, Verhoeven HM, *et al.* Pancytopenia due to high 6-methylmercaptopurine levels in a 6-mercaptopurine treated patient with Crohn's disease. Dig Liver Dis 2007; 39:182–186.
This paper describes the role of methylmercaptopurines in patients with pancytopenia due to mercaptopurine therapy.

38 Tay BS, Lilley RM, Murray AW, Atkinson MR. Inhibition of phosphoribosyl pyrophosphate amidotransferase from Ehrlich ascites-tumour cells by thiopurine nucleotides. Biochem Pharmacol 1969; 18:936–938.

39 • Zelinkova Z, Derijks LJ, Stokkers PC, *et al.* Inosine triphosphate pyrophosphatase and thiopurine s-methyltransferase genotypes relationship to azathioprine-induced myelosuppression. Clin Gastroenterol Hepatol 2006; 4:44–49.
The association between TPMT and ITPase polymorphisms and myelosuppression is investigated.

40 • Gardiner SJ, Gearry RB, Barclay ML, Begg EJ. Two cases of thiopurine methyltransferase (TPMT) deficiency: a lucky save and a near miss with azathioprine. Br J Clin Pharmacol 2006; 62:473–476.
In this short case report the clinical relevance of measuring for TPMT activity before starting mercaptopurine therapy is illustrated.

41 Armstrong VW, Shipkova M, von Ahsen N, Oellerich M. Analytic aspects of monitoring therapy with thiopurine medications. Ther Drug Monit 2004; 26:220–226.

42 Marinaki AM, Ansari A, Duley JA, *et al.* Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). Pharmacogenetics 2004; 14:181–187.

43 Maeda T, Sumi S, Ueta A, *et al.* Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. Mol Genet Metab 2005; 85:271–279.

44 • Shipkova M, Lorenz K, Oellerich M, *et al.* Measurement of erythrocyte inosine triphosphate pyrophosphohydrolase (ITPA) activity by HPLC and correlation of ITPA genotype–phenotype in a Caucasian population. Clin Chem 2006; 52:240–247.
Characteristics of the Caucasian population for ITPase are described in this paper.

45 • Bierau J, Bakker JA, Lindhout M, van Gennip AH. Determination of ITPase activity in erythrocyte lysates obtained for determination of TPMT activity. Nucleosides Nucleotides Nucleic Acids 2006; 25:1129–1132.
Methodological paper on the determination of ITPase in human erythrocyte lysate.

46 van Dieren JM, van Vuuren AJ, Kusters JG, *et al.* ITPA genotyping is not predictive for the development of side effects in AZA treated inflammatory bowel disease patients. Gut 2005; 54:1664.

47 Allorge D, Hamdan R, Broly F, *et al.* ITPA genotyping test does not improve detection of Crohn's disease patients at risk of azathioprine/6-mercaptopurine induced myelosuppression. Gut 2005; 54:565.

48 Marinaki AM, Duley JA, Arenas M, *et al.* Mutation in the ITPA gene predicts intolerance to azathioprine. Nucleosides Nucleotides Nucleic Acids 2004; 23:1393–1397.

49 von Ahsen N, Armstrong VW, Behrens C, *et al.* Association of inosine triphosphatase 94C>A and thiopurine S-methyltransferase deficiency with adverse events and study drop-outs under azathioprine therapy in a prospective Crohn disease study. Clin Chem 2005; 51:2282–2288.

50 Kerstens PJ, Stolk JN, De Abreu RA, *et al.* Azathioprine-related bone marrow toxicity and low activities of purine enzymes in patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:142–145.

51 Kerstens PJ, Stolk JN, Hilbrands LB, *et al.* 5-Nucleotidase and azathioprine-related bone-marrow toxicity. Lancet 1993; 342:1245–1246.

52 Goldsmith D, Carrey EA, Edbury S, *et al.* Mycophenolate mofetil, an inhibitor of inosine monophosphate dehydrogenase, causes a paradoxical elevation of GTP in erythrocytes of renal transplant patients. Clin Sci (Lond) 2004; 107:63–68.

53 Goldsmith DJ, Carrey EA, Edbury SM, *et al.* GTP concentrations are elevated in erythrocytes of renal transplant recipients when conventional immunosuppression is replaced by the inosine monophosphate dehydrogenase inhibitor mycophenolic acid mofetil (MMF). Nucleosides Nucleotides Nucleic Acids 2004; 23:1407–1409.

54 • Roberts RL, Gearry RB, Barclay ML, Kennedy MA. IMPDH1 promoter mutations in a patient exhibiting azathioprine resistance. Pharmacogenomics J 2006; 26 September [Epub ahead of print].
The first description of the role of IMPDH in mercaptopurine therapy.

55 Nakamura J, Lou L. Biochemical characterization of human GMP synthetase. J Biol Chem 1995; 270:7347–7353.

56 Anglicheau D, Sanquer S, Loriot MA, *et al.* Thiopurine methyltransferase activity: new conditions for reversed-phase high-performance liquid chromatographic assay without extraction and genotypic-phenotypic correlation. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 773:119–127.

57 •• van den Akker-van Marle ME, Gurwitz D, Detmar SB, *et al.* Cost-effectiveness of pharmacogenomics in clinical practice: a case study of thiopurine methyltransferase genotyping in acute lymphoblastic leukemia in Europe. Pharmacogenomics 2006; 7:783–792.
Study on the cost-effectiveness of pharmacogenomic analysis in several countries in the European Community.

58 •• Priest VL, Begg EJ, Gardiner SJ, *et al.* Pharmacoeconomic analyses of azathioprine, methotrexate and prospective pharmacogenetic testing for the management of inflammatory bowel disease. Pharmacoeconomics 2006; 24: 767–781.
A study on the cost-effectiveness of prospective testing of TPMT and clinical follow up in inflammatory bowel disease patients.

59 Winter J, Walker A, Shapiro D, *et al.* Cost-effectiveness of thiopurine methyltransferase genotype screening in patients about to commence azathioprine therapy for treatment of inflammatory bowel disease. Aliment Pharmacol Ther 2004; 20:593–599.

60 van Delden J, Bolt I, Kalis A, *et al.* Tailor-made pharmacotherapy: future developments and ethical challenges in the field of pharmacogenomics. Bioethics 2004; 18:303–321.

61 Gardiner SJ, Begg EJ. Pharmacogenetic testing for drug metabolizing enzymes: is it happening in practice? Pharmacogenet Genomics 2005; 15:365–369.