TPMT: thioguanine 1907/1908

ALL = acute lymphoblastic leukaemia, Cl_{or} = oral clearance, cytostat = cytostatic agent, IM = intermediate metaboliser (reduced TPMT enzyme activity; *1/variant), imm sup = immunosuppressant, MR = metabolic ratio, NM = normal metaboliser (normal TPMT enzyme activity; *1/*1), NS = non-significant, PM = poor metaboliser (low or absent TPMT enzyme activity; variant/variant), RBC = red blood cells, S = significant, TDM = therapeutic drug monitoring, 6-TG = thioguanine, 6-TGN = 6-thioguanine nucleotide, TPMT = thiopurine S-methyltransferase, UM = ultrarapid metaboliser (increased TPMT enzyme activity, not genetically determined), XO = xanthine oxidase

Disclaimer: The Pharmacogenetics Working Group of the KNMP formulates the optimal recommendations for each phenotype group based on the available evidence. If this optimal recommendation cannot be followed due to practical restrictions, e.g. therapeutic drug monitoring or a lower dose is not available, the health care professional should consider the next best option.

Brief summary and justification of choices:
TPMT converts thioguanine to inactive metabolites. The enzyme therefore reduces the percentage of thioguanine that is converted to the active metabolite.
Genetic variations in TPMT lead to decreased enzyme activity, which results in an increased percentage of thioguanine that is converted to the active metabolite. Therapeutic and toxic concentrations of the active metabolite are therefore reached at lower doses. If the dose is left unchanged, the risk of severe toxicity is higher for intermediate metabolisers (IM), and especially for poor metabolisers (PM) (de Hoogd 2019, van der Burg and Gerding 2018, Lennard 2015, Teml 2005, Herrlinger 2004, Standen 2001, and McBride 2000). In addition, at higher doses, these genetic variations increase the risk of tioguanine-induced sinusoidal obstruction syndrome (Stanulla 2021).
This is why the KNMP Pharmacogenetics Working Group decided that this concerns a gene-drug interaction and that action is required, namely to reduce the dose and/or to administer an alternative (yes/yes-interactions).
You can find a detailed overview of the observed clinical and kinetic effects per phenotype in the background information text of the gene-drug interactions in the KNMP Kennisbank. You may also have access to this background text via your pharmacy or physician electronic decision support system.
Substantiation of the (dose) recommendation for each phenotype is provided below.
<u>Justification of the (dose) recommendation per phenotype</u>

IM: A study including 40 IM patients showed that the median concentration of the active metabolite was 30% higher compared to NM patients, despite equal median doses (Lennard 2015). This is equivalent to a dose reduction to 77% to achieve the same median concentration of the active metabolite in IM patients as in NM patients at the standard dose. This was rounded off to 75% to be more achievable in clinical practice. This is high compared to the median found for mercaptopurine/azathioprine (50%), but it is similar to the mean found for these medicines (75%). For safety, the initial mercaptopurine/azathioprine dose should be 50% of the standard initial dose. As some IM tolerate the full dose, choosing an initial dose of 75% would also be justifiable. For this reason, and because thioguanine is often used as a last resort, the recommendation to reduce the initial dose to 75% of the standard initial dose is given despite the limited evidence. For oncolytics, toxicity and efficacy are strongly coupled, and it is unknown whether starting with a dose reduction based on genotype results in the same efficacy as reducing the dose based on toxicity. For this reason and because some IM tolerate the full dose, the KNMP Pharmacogenetics Working Group recommendation for these patients is to either start with 75% of the normal thioguanine dose or to start with the normal dose and reduce to 75% when adverse events necessitate a dose reduction. In determining the starting dose, next to the IM phenotype, the physician needs to take into account the comorbidity (e.g. the sensitivity for infections), the patient wishes (taking into account the above mentioned uncertainty) and the estimation of the aggression of the tumour (e.g. based on tumour genetics).

PM: The literature describes evidence of dose adjustment in 2 PM patients (to 7.14% and 6.25% respectively; mean 6.7%) (Lennard 2015 and Mares 2009). In addition, a patient on 1.07 times the normal 3.0 mg/kg dose developed 6-TGN concentrations 10.5 times the upper limit of the therapeutic range (de Hoogd 2019). This indicates that the dose in this patient should have been less than 10.2% of the normal dose in order for the 6-TGN levels to not exceed the upper limit of the therapeutic range. The levels found are consistent with the levels found for mercaptopurine/azathioprine (10%) considering that these medicines can be administered at relatively higher doses in patients with reduced TPMT activity, because these medicines are converted by

TPMT to metabolites that contribute to toxicity. For this reason, and because thioguanine is often used as a last resort, the recommendation to reduce the initial dose to 6-7% of the standard initial dose is given despite the limited evidence. The option of choosing an alternative is also included in the recommendation.

Recommendation concerning pre-emptive genotyping, including justification of choices:
The Dutch Pharmacogenetics Working Group considers genotyping before starting thioguanine to be essential for drug safety. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection.
Data in humans are scarce for thioguanine, but extrapolation of data from azathioprine and 6-mercaptopurine was considered to be justified (see Brief summary and justification of choices above for the similar effects of TPMT IM and PM on dose requirement of thioguanine and azathioprine/6-mercaptopurine). For this reason, for determination of the clinical implication score for thioguanine, the evidence supporting the severity of the clinical effect and the number needed to genotype to prevent one patient developing an adverse event grade ≥ 3 were derived from azathioprine/6-mercaptopurine. This resulted in the clinical implication of the TPMT-thioguanine interaction scoring 7 out of the maximum of 10 points (with pre-emptive genotyping considered to be essential for scores ranging from 6 to 10 points):
A case of unsuspected, possibly life-threatening myelosuppression has been observed in a PM (code F corresponding to grade 5) (McBride 2000). This results in the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for CTCAE grade 5).
The increased risk for serious toxicity (code D-E corresponding to grade 3-4) has been shown in only 1 study for thioguanine, but in 3 studies and 1 systematic review for azathioprine and 6-mercaptopurine. This results in the maximum score of 3 points for the second criterion of the clinical implication score, the level of evidence supporting the associated clinical effect grade ≥ 3 (3 points for three or more publications with level of evidence score ≥ 3).
The number needed to genotype cannot be deduced from the literature for thioguanine. For azathioprine/6-mercaptopurine, this number was deduced from the prevalence of PM, because almost all PM develop severe leukopenia and intolerance on normal thiopurine doses. The frequencies of the *2-, *3A-, *3B- and *3C-alleles in the Netherlands are 0.4, 3.5, 0.4 and 0.8% respectively, corresponding to a PM frequency of 0.26%. This would amount to a number needed to genotype to find one PM, and thus one patient developing an adverse event grade ≥ 3 on normal therapy, of 384. The calculated number needed to genotype of 384 results in 1 out of the maximum of 3 points (1 point for 100 < NNG ≤ 1000).
The Dutch Summary of Product Characteristics (SmPC) indicates that PM have an increased risk for severe toxicity. This results in 1 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (1 point for at least one genotype/phenotype mentioned in the SmPC, but not mentioned as a contra-indication and no recommendation to genotype).
Despite genotyping before starting thiopurine treatment scoring as essential for drug safety, results from 12 cost-effectiveness analyses for azathioprine or 6-mercaptopurine are inconclusive and tend to point to a lack of cost-effectiveness. However, the 3 most recent cost-effectiveness analyses suggested cost-effectiveness. For thioguanine, one of these recent cost-effectiveness analyses calculated the cost per death averted by TPMT genotyping and TPMT-guided therapy to be € 385,084. With a threshold of additional costs of € 20,000-60,000 per quality-adjusted life year (QALY) gained, this would be cost-effective if patients lived afterwards for 6-19 years with optimal quality of life.

The table below uses the KNMP nomenclature for NM, PM and IM. As a result, the definitions of NM, PM and IM in the table below can differ from the definitions used by the authors in the article.

Before 2011, articles with the indication cytostatic therapy were included first, followed by articles with the indication immune suppression. Within each group, articles were included in order of publication date (most recent first). From 2011, articles were included in order of publication date only.

Source	Code	Effect	Comments
ref. 1, imm sup Bayoumy AB et al. Relationship between thiopurine S-methyltransferase genotype/phenotype and 6-thioguanine nucleotide levels in 316 patients with inflammatory bowel	3	71 patients with inflammatory bowel disease were treated with thioguanine (median dose 20 mg/day, range 10-40 mg/day) for a mean of 21.1 months. Patients with 6-TGN levels below 100 pmol/8x10^8 red blood cells were excluded to reduce the influence of noncompliance. Only patients using thioguanine as monotherapy or as concomitant therapy with biologicals were included. So, aminosalicylates were excluded. However, other TPMT inhibitors were not excluded.	Authors' conclusion: "Our findings indicated that TPMT measurements at thioguanine initiation can be useful but are not necessary for daily practice. TPMT geno-

<table>
<tr>
<td>disease on 6-thioguanine.
Ther Drug Monit 2021;43:617-623.
PMID: 34521801.

ref. 1, continuation</td>
<td>IM: A</td>
<td>Genotyping:
- 67x NM
- 4x IM

Results:
<table>
<tr><td colspan="2">6-TGN level compared to NM (6-TGN level of 467.5 pmol/8x10^8 red blood cells):</td></tr>
<tr><td>IM</td><td>x 2.4</td></tr>
</table>

Note: There was no relationship with 6-TGN level and laboratory parameters (including blood counts and liver enzymes) in a larger group including also 245 non-genotyped patients.

NOTE: The alleles determined were not stated. However, *2 and *3A were found, indicating that genotyping was performed for the most important gene variants in this Dutch population.</td>
<td>types and phenotypes are both associated with significant differences in 6-TGN levels between metabolic groups. However, the advantage of thioguanine remains that RBC 6-TGN measurements are not crucial to monitor treatments in patients with IBD because these measurements did not correlate with laboratory result abnormalities.”</td>
</tr>
<tr>
<td>ref. 2, cytostat
Stanulla M et al.
Hepatic sinusoidal obstruction syndrome and short-term application of 6-thioguanine in pediatric acute lymphoblastic leukemia.
Leukemia 2021;35:2650-7.
PMID: 33714975.</td>
<td>3</td>
<td>13 paediatric patients with acute lymphatic leukaemia developing hepatic sinusoidal obstruction syndrome severe enough to be classified as severe adverse event after being treated once or twice with late-intensification protocols containing a 2-week treatment with thiopurine 60 mg/m^2 per day were compared to a subgroup of 813 genotyped paediatric patients without sinusoidal obstructive syndrome classified as severe adverse event treated according to the same protocol simultaneously.
In addition, development of sinusoidal obstruction syndrome severe enough to be classified as severe adverse event was analysed in a cohort of 1566 paediatric patients treated once with a late-intensification protocol containing a 2-week treatment with thiopurine 60 mg/m^2 per day.
A severe adverse event was defined as any untoward medical occurrence that (1) resulted in death; (2) was life threatening (defined as an event in which the patient was at risk of death at the time of the event; it did not refer to an event which hypothetically might have caused death if it would have been more severe); (3) required or prolonged hospitalization; (4) resulted in persistent or significant disability/incapacity; (5) was a congenital anomaly/birth defect in the offspring.
The prevalence of thioguanine-induced sinusoidal obstruction syndrome in the first cohort (including the non-genotyped patients) was 0.33%. The prevalence of thioguanine-induced sinusoidal obstruction syndrome in the second cohort was 0.57%. All sinusoidal obstruction syndrome cases in the first cohort were moderate grade according to Ponte di Legno criteria (i.e. bilirubin 103–342 µmol/L and/or weight gain more than 5% or ascites). They all required treatment of sinusoidal obstruction syndrome, but recovered without residual symptoms such as portal hypertension/splenomegaly or nodular regenerative hyperplasia on follow-up, and achieved continuous complete remission of acute lymphatic leukaemia. In neither cohort, a PM had sinusoidal obstruction syndrome severe enough to be classified as severe adverse event.
Patients developing sinusoidal obstruction syndrome in association with hematopoietic stem cell transplantation were excluded, but comedication affecting TPMT was not. In addition, in the first cohort, 4 patients developed sinusoidal</td>
<td>Authors’ conclusion:
“Hepatic sinusoidal obstruction syndrome is associated with short-term exposure to 6-thioguanine during treatment of pediatric ALL and sinusoidal obstruction syndrome risk is increased for patients with low-activity TPMT genotypes.”</td>
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</table>

<table>
<tr>
<td>ref. 2, continuation</td>
<td>IM: C</td>
<td>obstruction syndrome in the induction phase, i.e. prior to thioguanine administration, so comedication inducing sinusoidal obstruction syndrome was not fully excluded. Neither was sinusoidal obstruction syndrome developing prior to thioguanine administration in the second cohort.

Genotyping:
cases: - 5x NM - 8x IM
genotyped control group: - 755x NM - 54x IM - 4x PM
second cohort: - 755x NM - 54x IM - 4x PM

Results:
<table>
<tr><td colspan="3">Risk of thioguanine-induced sinusoidal obstruction syndrome for IM compared to NM:</td></tr>
<tr><td></td><td></td><td>value for NM</td></tr>
<tr><td>first cohort (cases and genotyped control group)</td><td>OR = 22.4 (95% CI: 7.1-70.7) (S)</td><td>0.14%</td></tr>
<tr><td>second cohort</td><td>RR = 6.73 (95% CI: 1.71-26.53) (S)</td><td>0.41%</td></tr>
</table>
NOTE: Genotyping was for *2 and *3. These are the most important gene variants in this German population.</td>
<td></td>
</tr>
<tr>
<td>ref. 3 – imm sup
de Hoogd S et al. Severe pancytopenia and aspergillosis caused by thioguanine in a thiopurine S-methyltransferase deficient patient: a case report. Eur J Gastroenterol Hepatol 2019;31:1592-6. PMID: 31464791.</td>
<td>2

PM: E</td>
<td>In a 50-year old woman with Crohn’s disease and a history of azathioprine intolerance, treatment with thioguanine 25 mg/ day (0.32 mg/kg) was stopped after 4 weeks due to high 6-TGN levels (10.5 times the upper limit of the proposed therapeutic range) accompanied by a low haemoglobin (10.8 g/dl) and leukocyte count (2.1 × 10^9/L). Four days after discontinuation, the patient was hospitalised with a severe pancytopenia (leukocyte count 1.0x10^9/L; thrombocyte count 6x10^9/L; haemoglobin 6.4 g/dl) and increased C-reactive protein, with clinical symptoms of a neutropenic fever with an infectious bronchiolitis, which was identified as angioinvasive aspergillosis later. 14 days after thioguanine discontinuation, the 6-TGN level was still 2.9 times the upper limit of the proposed therapeutic range, with a persistent pancytopenia (leukocyte count 1.3x10^9/L; lymphocyte count 1.03x10^9/L; monocyte count 0.05x10^9/L; thrombocyte count 6x10^9/L; haemoglobin 6.0 mmol/L). Fifteen days after hospital admission, blood count started to recover with granulocyte colony stimulating factor (G-CSF) therapy. There were no residual symptoms of the aspergillosis after 6 weeks of voriconazole treatment. One year after hospital admission, nodular regenerative hyperplasia due to the relatively toxic dose of thioguanine was ruled out.
Genotyping after the discontinuation of thioguanine showed the patient to be PM (*3A/*3A).</td>
<td>Authors’ conclusion:
‘Clinicians should be aware of the impact of TPMT deficiency on the metabolism of thioguanine and should consider performing pre-emptive TPMT genotyping in combination with frequent blood test monitoring when using thiopurines in general.’

6-TGN concentration at 25 mg (0.32 mg/ kg) per day compared to the upper limit of the therapeutic range: PM: 1050%</td>
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<td>ref. 4, imm sup
van der Burg M and Gerding MN. [Pancytopenia associated with thioguanine use]. Ned Tijdschr Geneeskd</td>
<td>1</td>
<td>In a 56-year old man with colitis ulcerosa, treatment with thioguanine was stopped after 3 weeks due to infection-like symptoms. Pancytopenia was diagnosed 1 week later.
The patient did not use corticosteroids or biologicals, but the authors do not mention or exclude other co-medication.
The medical history of the patient reported development of leukopenia when treated with 6-mercaptopurine earlier. After cessation of 6-mercaptopurine, leukocyte count gradually</td>
<td>Authors’ conclusion:
‘For patients who previously developed leukopenia when treated with azathioprine or mercaptopurine,</td>
</tr>
</table>

2018;162:D2839. PubMed PMID: 30379500. **ref. 4, continuation**	PM: E	restored. Results: - 1 week after cessation of thioguanine, his haemoglobin was 4.4 mmol/l (normal range: 8.5-11.0 mmol/l), his leukocyte count 1.2 x 10^9/L (leukopenia grade 3), and his thrombocyte count 5 x 10^9/L (thrombocytopenia grade 4) - after diagnosis of pancytopenia, patient received 2 units of erythrocytes on day 1 and 2, 1 unit of erythrocytes on day 8, 12 and 16, and 1 unit of thrombocytes on day 1 and 8. Leukocyte count was restored to normal values at day 38 after diagnosis, haemoglobin at day 64, and thrombocyte count was still not within normal values at day 64. Thrombocytopenia reduced from grade 4 to grade 3 for longer than one day at day 27 after diagnosis and to grade 1 at day 23. Patient finally recovered fully. - 10 days after cessation of thioguanine, 6-TGN levels were still too high: 3800 pmol/8x10^8 RBC (normal values: 600-2600 pmol/8x10^8 RBC) - genotyping showed the patient to be *3A/*3A Note: The authors indicated that there is no consensus in the Dutch guideline for inflammatory bowel disease about TPMT genotyping before starting a thiopurine (Handleiding behandeling IBD - 2014-2015. Moderniseren van de richtlijn IBD 2009. https://www.icc-ibd.com/upload/files/DocumentvolledigHandleidingmetliteratuurvs7.21.pdf). In addition, the authors indicated that in only 25% of the patients with Crohn's disease bone marrow suppression can be explained by a decreased TPMT enzyme activity (Bär F et al. Thiopurines in inflammatory bowel disease revisited. World J Gastroenterol 2013;19:1699-706). Moreover, the authors indicated that the Dutch centre for registration of adverse events (Lareb) reported 77 adverse events of thioguanine, of which 20 (26%) were hematologic. Of these 20 patients, 4 developed bone marrow failure (5.2% of the total number of patients) and 1 developed pancytopenia (1.3%).	additional vigilance is required if thioguanine treatment is considered; TPMT genotyping is recommended in these patients.'
ref. 5, cytostat McAtee CL et al. Treatment-related sinusoidal obstruction syndrome in children with de novo acute lymphoblastic leukemia during intensification. Cancer Chemother Pharmacol 2017;80:1261-4. PubMed PMID: 29051993.	3	8 children were analysed who developed sinusoidal obstruction syndrome (veno-occlusive disease) within 60 days of a 14-day course of thioguanine 60 mg/m^2 per day as part of a delayed intensification treatment for acute lymphoblastic leukaemia (on day 29-42 of the 8-week delayed intensification protocol). Of the total number of treated patients, 1.5% developed sinusoidal obstruction syndrome. 20% of the patients with sinusoidal obstruction syndrome died, while the other 80% fully recovered. Relevant co-medication was not excluded. Dexamethasone, vincristine, and pegylated asparaginase, all administered in the first four weeks of the delayed intensification treatment, have been suggested to contribute to thioguanine-induced hepatotoxicity. However, all patients received these co-medications. Results: 63% of patients with sinusoidal obstruction syndrome was IM (*1/*3A), and 37% NM. The authors did not	Authors' conclusion: 'Intermediate thiopurine methyltransferase genotype was noted in 5/8 patients with data available.'

ref. 5, continuation	IM: AA	compare these prevalences with those in patients without sinusoidal obstruction syndrome or with those in the general population (NS). NOTE: The authors indicated that sinusoidal obstruction syndrome after delayed intensification therapy differed in several respects from sinusoidal obstruction syndrome after maintenance therapy: - early onset after or during a short course (within 4 weeks after a 14 days course) versus onset after long-term treatment (typically several months after start of treatment) - low incidence (0.3-1.6%) versus high incidence (11-20%) - high mortality rate (20%) versus low mortality rate (< 0.4%) - generally brief hepatosplenomegaly and thrombocytopenia in surviving patients (median of 3 weeks) versus persistent splenomegaly and thrombocytopenia in 25% of patients (median of 39 months after cessation of thioguanine) Sinusoidal obstruction syndrome after delayed intensification therapy with thioguanine was more similar to sinusoidal obstruction syndrome after hematopoietic stem cell transplantation than sinusoidal obstruction syndrome after maintenance therapy with thioguanine. NOTE: Genotyping was for *3A. This is the most important gene variant in this population from the USA. The authors did not specify which variants were genotyped, so it is not known whether additional variants were determined.	
ref. 6 - cytostat Lennard L et al. Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: the influence of thiopurine methyltransferase pharmacogenetics Br J Haematol 2015;169:228-40. PubMed PMID: 25441457.	3 IM: E	426 children with acute lymphoblastic leukaemia were treated with thioguanine for 2 years. The initial dose was 40 mg/m^2 for NM and IM, and 4.0 mg/m^2 for PM. Thioguanine was administered in combination with methotrexate, vincristine and either dexamethasone or prednisone. Relevant co-medication was not excluded. Clinical outcome measures were only determined in combination with a group receiving mercaptopurine as the thiopurine (n = 709) and were available to 61% of the patients. Genotyping (thioguanine only): - 385x NM (*1/*1) - 40x IM (1x *1/*2, 33x *1/*3A, 4x *1/*3C, 1x *1/*21, 1x *1/*34) - 1x PM (*3A/*3A) IM versus NM: Mercaptopurine or thioguanine: - Duration of cytopenia-induced thiopurine dose interruptions increased by 34% (from 15.5% to 20.8% of the total duration) (S) - Neutropenia increased by 8.1% (from 23.4% to 25.3% of the total duration) (S) - Thrombocytopenia increased by 159% (from 3.4% to 8.8% of the total duration) (S) - The mean daily thiopurine dose decreased by 10% (from 78.0% to 70.4% of the initial dose for NM/IM) (S) - 5-year EFS (event-free survival (EFS), with an event defined as time to relapse or death) increased by 10% for *1/*3A versus NM (from 80% to 88%) (S), but multivariate regression analysis did not identify a significantly decreased risk of relapse or death for all IM patients apart from those with *1/*3C (NS)	Authors' conclusion: "TPMT*1/*3A heterozygotes had a better event-free survival than TPMT wild-type patients. Thiopurine induced cytopenias were not detrimental to treatment outcome. .... The TPMT heterozygotes tolerated significantly lower average % dosages than the TPMT wild-type patients (70% vs 78% for TPMT wild-type, a daily-dose difference of 3.2 mg/m^2 per day thioguanine). However, the range of thiopurine doses tolerated was wide, with the upper and lower limits similar for both TPMT genotypes. These findings do not

ref. 6, continuation	PM: A (2)	- 5-year EFS decreased by 34% in *1/*3C patients versus NM patients (from 80% to 53%) (S), and multivariate regression analysis showed an increased risk of relapse or death (HR = 3.2; 95% CI: 1.5-6.8) (S) There was no difference between *1/*3C and *1/*3A in mean daily dose or incidence of cytopenia. However, there was evidence of poor compliance in the mercaptopurine group. - No difference in secondary cancers (median follow-up 11.3 years) (NS) Thioguanine only: - Increase in the median 6-TGN concentration by 30% (from 1904 to 2468 pmol/8x10^8 RBC) (S) measured at the same median dose (40 mg/m^2) (NS) PM versus NM: Thioguanine only: - The eventual dose was 6.25% of the dose in NM patients (2.5 mg/m^2). - At this dose, the 6-TGN concentration was 1.2-fold higher than the median 6-TGN concentration for NM (2252 and 1904 pmol/8x10^8 RBC respectively). NOTE: Genotyping was performed for *2, *3A, *3B and *3C. Exons 3 to 10 were sequenced to identify new or rare variants (*9, *21, *32-*34).	support any change in the prescribing criteria (both genotypes start at the same standard protocol dose and titrate to toxicity)." Median 6-TGN concentration versus NM: IM: 130% Dose versus NM: PM: 6.25%
ref. 7 - cytostat Wray L et al. TPMT and MTHFR genotype is not associated with altered risk of thioguanine-related sinusoidal obstruction syndrome in pediatric acute lymphoblastic leukemia: a report from the Children's Oncology Group. Pediatr Blood Cancer 2014;61:2086-8. PubMed PMID: 24737678.	3 IM+PM: AA	340 children with acute lymphoblastic leukaemia were treated with thioguanine 50-60 mg/m^2. Two different protocols were used for post-induction therapy. Relevant co-medication was not excluded. Sinusoidal obstruction syndrome is chemotherapy-induced hepatic veno-occlusive disease. It occurred in 22.5% of the patients. Genotyping: - *3A: 286x NM, 54x IM+PM. (Patients in whom one of the two polymorphisms could not be identified were assumed to be wild-type.) - *3B: 256x NM, 35x IM+PM (The genotype was unknown for 49 patients.) - *3C: 302x NM, 31x IM+PM (The genotype was unknown for 7 patients.) Results: - None of the alleles *3A, *3B and *3C were associated with a risk of sinusoidal obstruction syndrome (NS) NOTE 1: The definition of sinusoidal obstruction syndrome was less strict in this study than in other studies. The data generated by this study therefore do not rule out that the TPMT genotype plays a part in determining the risk of severe sinusoidal obstruction syndrome. NOTE 2: Genotyping was performed for *3A, *3B and *3C. DNA for genotyping was obtained from bone marrow in remission. All genotypes were in Hardy-Weinberg equilibrium.	Authors' conclusion: "TPMT and MTHFR C677T genotypes were not associated with sinusoidal obstruction syndrome risk."
ref. 8 - cytostat Lennard L et al. The thiopurine methyltransferase genetic polymorphism is associated	3	1492 children with ALL were randomised to maintenance therapy with either thioguanine at an initial dose of 40 mg/m^2/day (n=748) or 6-mercaptopurine at an initial dose of 75 mg/m^2/day (n=744). The thiopurine dose was titrated to toxicity guided by neutrophil and platelet counts. Co-medication: non-relevant cytostatic agents and steroids.	Authors' conclusion: "Thioguanine was associated with liver damage in 11% of children

with thioguanine-related veno-occlusive disease of the liver in children with acute lymphoblastic leukemia. Clin Pharmacol Ther 2006;80:375-83. **ref. 8, continuation**	IM: AA	Patients with thioguanine-related hepatic veno-occlusive disease (sinusoidal obstruction syndrome) compared to a control group without veno-occlusive disease: - TPMT activity decreased from median 15.2 U to 13.4 U (S by 12%). - The percentage of IM phenotype increased from 11% to 23% (S by 109%) - The percentage of IM genotype increased from 9.8% to 18% (NS by 84%) - The 6-TGN concentrations at a 6-TG dose of 40 mg/m^2/day increased from median 1916 to 2034 pmol/8x10^8 RBC (NS by 6%) Patients with persistent thioguanine-related splenomegaly due to portal hypertension compared to a control group without splenomegaly: - TPMT activity decreased from median 15.5 U to 13.9 U (S by 10%). - No difference in 6-TGN concentrations at a 6-TG dose of 40 mg/m^2/day. There was a negative correlation between TPMT activity and 6-TGN concentrations (S). NOTE: Genotyping was only performed for *3 alleles, not for *2 alleles.	randomized to thioguanine without an improvement in event-free survival rate. The association of lower TPMT activity with thioguanine-related liver damage could provide a means of identifying at-risk patients."
ref. 9 - cytostat Standen GR et al. Heterozygosity for the thiopurine methyltransferase *3A allele in an acute non-lymphoblastic leukaemia patient with delayed marrow regeneration following H-DAT chemotherapy. Br J Haematol 2001;112:1089.	2 IM: C	Patient with acute non-lymphoblastic leukaemia received thioguanine 100 mg/m^2 twice daily for two cycles of 10 and 8 days respectively. Co-medication: non-relevant cytostatic agents. The blood counts recovered significantly more slowly after the second cycle. A bone marrow biopsy on day 40 showed distinct hypocellularity. Neutropenia recovered on Day 45, but the platelet count was still <100x10^9/L on Day 80 and the patient still required RBC transfusions. The patient was found to be an IM (*1/*3A).	Authors' conclusion: "The clinical course of our patient raises the possibility that TPMT mutations might also influence thioguanine toxicity in patients with ANLL. Pharmacogenetic factors could be particularly important when this agent is included in regimes that approach maximum haemopoietic tolerance."
ref. 10 - cytostat McBride KL et al. Severe 6-thioguanine-induced marrow aplasia in a child with acute lymphoblastic leukemia and inherited thiopurine methyltrans-ferase deficiency. J Pediatr Hematol Oncol 2000;22:441-5.	2 PM: F	An eight-year-old boy with ALL received consolidation therapy of thioguanine 50 mg/m^2/day. Co-medication: non-relevant cytostatic agents, immunosuppressants and antibiotics. The patient developed severe and prolonged pancytopenia. The bone marrow plasma cell percentage had decreased to 5%. The patient had neutropenia for 67 days, and his anaemia and thrombocytopenia only started to recover after 96 days. Daily platelet transfusions were needed. The patient was found to be a PM (*3A/*3A). Thioguanine therapy was not resumed.	Authors' conclusion: "We report the first case of severe and prolonged pancytopenia caused by 6-thioguanine in an 8-year-old boy with ALL and inherited TPMT deficiency. To obviate this life-threatening complication, clinicians should consider assaying TPMT activity before

ref. 10, continuation			initiating therapy with 6MP and, particularly, 6TG in children with ALL."
ref. 11 – imm supp Mares WG et al. Safe 6-thioguanine therapy of a TPMT deficient Crohn's disease patient by using therapeutic drug monitoring. J Crohns Colitis 2009;3:128-30.	2 PM: A	Infliximab therapy was supplemented with thioguanine in a 34-year-old patient with Crohn's disease and fistula formation. As phenotyping had shown that the patient was a PM, low-dose thioguanine was used. At a dose of 20 mg/week (0.036 mg/kg/day), the 6-TGN concentration increased to 1003 pmol/ $8x10^8$ RBC in the course of 3 weeks without myelosuppression. After dose reduction to 20 mg/2 weeks (0.018 mg/kg/day), the 6-TGN concentrations remained between 500 and 900 pmol/$8x10^8$ RBC. Crohn's disease was in remission and the patient's blood cell counts and liver tests remained normal (current therapy duration: 30 months). The patient refused a liver biopsy and nodular regenerative hyperplasia could therefore not be excluded. The authors concluded that an optimal dose of thioguanine could not be established in patients with Crohn's disease or ulcerative colitis. Derijks et al., 2003 found 6-TGN concentrations of 937 ± 325 pmol/$8x10^8$ RBC when 19 patients were treated with thioguanine 20 mg/day. The authors reported that studies show evidence that thioguanine-induced hepatotoxicity (especially nodular regenerative hyperplasia and veno-occlusive disease) are dose-dependent and seem to be associated with 6-TGN concentrations > 1000 pmol/$8x10^8$ RBC.	Authors' conclusion: "Our case demonstrates that very low dose 6-TG under close clinical surveillance and frequent therapeutic drug monitoring, may be a rescue drug for IBD-patients with low or without functional TPMT activity." Dose versus NM: PM: 7.14%
ref. 12 – imm supp Teml A et al. A prospective, open-label trial of 6-thioguanine in patients with ulcerative or indeterminate colitis. Scand J Gastroenterol 2005;40:1205-13.	3* IM: C (2)	16 patients with inflammatory bowel disease and intolerance or resistance to azathioprine/6-mercaptopurine (15x NM, 2x IM (*1/*3A)) were treated with thioguanine for 26 weeks in a prospective open-label study. Thioguanine dose: 20 mg/day for 2 weeks, followed by 40 mg/day, and increased up to 80 mg/day after ≥ 8 weeks if needed (3 NM: up to 60 mg/day, 1 NM: up to 80 mg/day); adverse-event-related dose reductions were permitted. Co-medication: mesalazines (n=5, dose not known), non-relevant immunosuppressants. Smoking reported. - Fourteen adverse events were observed in the 16 patients. - One patient (NM) developed serious adverse events that required hospitalisation and withdrawal of therapy. - One IM patient with low body weight (38 kg) had hair loss despite a dose reduction to 30 mg/day. This resolved after reduction to 20 mg/day. - The other IM patient developed arthralgia/myalgia at a 40 mg/day dose. This did not improve following dose reduction and therapy was discontinued.	Authors' conclusion: "In the present study, TPMT did not help in explaining 6-TG-related side effects."
ref. 13 – imm supp Herrlinger KR et al. Thioguanine nucleotides do not predict efficacy of tioguanine in Crohn's disease. Aliment Pharmacol Ther 2004;19:1269-76.	3* IM: D (2)	26 patients with Crohn's disease (25x NM, 1x IM (*1S/*3A)) were treated with thioguanine ≥ 40 mg/day for 24 weeks. At week 12, the dose was increased to 80 mg/day in 10 patients not in remission, including the IM patient. Co-medication: mesalazines (frequency and dose not known), steroids. - Toxicity occurred in 3 patients: 2 NM (abnormal liver tests that recovered without dose reduction and mild leukopenia) and the IM (signs of myelotoxicity: mild leukopenia, thrombocytopenia and anaemia). The two NM patients did not have 6-TGN concentrations above the median; the IM patient using thioguanine 80 mg/day had a very high 6-TGN concentration (4665 pmol/$8x10^8$ RBC versus the average of 1660	Authors' conclusion: "Dose escalation to 80 mg was tolerated well in all patients except in one subject who was an intermediate methylator and consequently developed excessive 6-TGN levels resulting in bone

ref. 13, continuation		pmol/8x10^8 RBC in a group of 9x NM and 1x IM).	marrow depression."
ref. 14 - cytostat SmPC Lanvis (thioguanine) 08-06-18.	0 PM: E	Dose: Patients with an inherited low activity or absence of activity of thiopurine S-methyltransferase (TPMT), receiving conventional doses of thioguanine, have an increased risk for severe toxicity. Doses in these patients should generally be reduced substantially. The optimal starting dose for homozygous deficient patients has not been established. Most patients with heterozygous TPMT deficiency can tolerate the recommended thioguanine doses, but in some patients a dose reduction may be required. Genotypic and phenotypic TPMT tests are available. Warnings: If administration of thioguanine is stopped in time, bone marrow suppression is reversible. Patients with an inherited deficiency of the TPMT enzyme may be unusually sensitive to the myelosuppressive effect of thioguanine and may be prone to developing bone marrow depression shortly after initiation of treatment with thioguanine. Concomitant administration of TPMT inhibiting drugs, such as olsalazine, mesalazine or sulphasalazine, can exacerbate the bone marrow suppression. Some laboratories offer testing for TPMT deficiency, but these tests cannot identify all patients at risk of severe toxicity. Therefore close monitoring of blood counts is still necessary. Pharmacodynamics: Patients with variants in both the NUDT15 and the TPMT enzyme have significantly less thiopurine tolerance than patients with risk alleles in only one of these two genes.	
ref. 15 - cytostat SmPC Tabloid (thioguanine), USA, 23-05-18.	0 PM: E	Clinical pharmacology: Several published studies indicate that patients with reduced TPMT or NUDT15 activity receiving usual doses of mercaptopurine, accumulate excessive cellular concentrations of active 6-TGNs, and are at higher risk for severe myelosuppression. In a study of 1028 children with ALL, the approximate tolerated mercaptopurine dosage range for patients with TPMT and/or NUDT15 deficiency on mercaptopurine maintenance therapy (as a percentage of the planned dosage) was as follows: heterozygous for either TPMT or NUDT15, 50-90%; heterozygous for both TPMT and NUDT15, 30-50%; homozygous for either TPMT or NUDT15, 5-10%. Approximately 0.3% (1:300) of patients of European or African ancestry have two loss-of-function alleles of the TPMT gene and have little or no TMPT activity (homozygous deficient or poor metabolisers), and approximately 10% of patients have one loss-of-function TPMT allele leading to intermediate TPMT activity (heterozygous deficient or intermediate metabolisers). The TPMT*2, TPMT*3A, and TPMT*3C alleles account for about 95% of individuals with reduced levels of TPMT activity. Warnings: Evaluate patients with repeated severe myelosuppression for thiopurine S-methyltransferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency. TPMT genotyping or phenotyping (red blood cell TPMT activity) and NUDT15 genotyping can identify patients who have reduced activity of these enzymes. Patients with homozygous TPMT or NUDT15 deficiency require substantial dosage reductions. Bone marrow suppres-	

ref. 15, continuation		sion could be exacerbated by co-administration with drugs that inhibit TPMT, such as olsalazine, mesalazine, or sulphasalazine. Precautions: Consider testing for TPMT and NUDT15 deficiency in patients who experience severe bone marrow toxicities or repeated episodes of myelosuppression. Dose: Patients with homozygous deficiency of either TPMT or NUDT15 enzyme typically require 10% or less of the standard thioguanine dosage. Reduce initial dosage in patients who are known to have homozygous TPMT or NUDT15 deficiency. Most patients with heterozygous TPMT or NUDT15 deficiency tolerate recommended thioguanine doses, but some require dose reduction based on toxicities. Patients who are heterozygous for both TPMT and NUDT15 may require more substantial dosage reductions. Reduce the dosage based on tolerability.	

[#] For studies that did not show significant differences for IM due to very low numbers of IM in the study (≤ 2), the effect for IM was scored as if this concerned a case. This was indicated by placing the case code (2) behind the score.

Risk group	Use of TPMT inhibitors (aminosalicylates: mesalazine, olsalazine or sulphasalazine, furosemide, acetylsalicylic acid), use of inhibitors of *de novo* purine synthesis (methotrexate), NUDT15 IM or PM (frequent in East Asian patients) Note: results regarding the effect of the aminosalicylates on thiopurines are contradictory. Five studies clearly showed no *in vivo* drug interaction (Szumlanski CL et al. Sulphasalazine inhibition of thiopurine methyltransferase: possible mechanism for interaction with 6-mercaptopurine and azathioprine. Br J Clin Pharmacol 1995;39:456-9; Dewit O et al. Interaction between azathioprine and aminosalicylates: an in vivo study in patients with Crohn's disease. Aliment Pharmacol Ther 2002;16:79-85; Dilger K et al. Monitoring of thiopurine methyltransferase activity in postsurgical patients with Crohn's disease during 1 year of treatment with azathioprine or mesalazine. Ther Drug Monit 2007;29:1-5; de Graaff P et al. Influence of 5-aminosalicylic acid on 6-thioguanosine phosphate metabolite levels: a prospective study in patients under steady thiopurine therapy. Br J Pharmacol 2010;160: 1083-91; Reinisch W et al. Azathioprine versus mesalazine for prevention of postoperative clinical recurrence in patients with Crohn's disease with endoscopic recurrence: efficacy and safety results of a randomised, double-blind, double-dummy, multicentre trial. Gut 2010;59:752-9).

Comments:

- FDA recommendations. The FDA recommendations have been taken from the American authorisation file on Tabloid Brand Thioguanine (thioguanine). This authorisation file does not contain additional information compared to the Dutch authorisation file of Lanvis (thioguanine).
- Dose recommendations in reviews/articles:
  - Clinical Pharmacogenetics Implementation Consortium Guidelines (Relling et al., Clin Pharmacol Ther 2011;89:387-91, Clin Pharmacol Ther 2013;93:324-5, and Clin Pharmacol Ther 2019;105:1095-1105. PubMed PMID: 30447069):
    CPIC defines TPMT IM and TPMT PM as we do (one or two no function alleles, respectively), but considers only *2, *3A, *3B, *3C, *4, *11, *14, *15, *23, and *29 to be no function alleles. CPIC considers the other alleles, including *5 through *10 and *12 to be alleles with uncertain function. CPIC groups combinations of one allele with uncertain function and one no function allele in the phenotype ‘possible IM’ instead of in the IM phenotype. In addition, CPIC groups combinations of two uncertain function alleles and combinations of one normal function and one uncertain function allele in the phenotype ‘indeterminate’.
    CPIC indicates that thioguanine is mainly used for myeloid leukaemia. CPIC did not perform a literature review for thioguanine separately, but only for all thiopurines together. As a consequence, the thioguanine recommendations are mainly based on data on azathioprine/6-mercaptopurine.

CPIC indicates, that although there is lower affinity between thioguanine and TPMT than between 6-mercaptopurine and TPMT, TPMT significantly affects thioguanine pharmacokinetics and its cytotoxic effects (McBride 2000; Hosni-Ahmed A et al. Thiopurine methyltransferase predicts the extent of cytotoxicity and DNA damage in astroglial cells after thioguanine exposure. PLoS One 2011;6:e29163; Higgs JE et al. Are patients with intermediate TPMT activity at increased risk of myelosuppression when taking thiopurine medications? Pharmacogenomics 2010;11:177-88; Hartford C et al. Differential effects of targeted disruption of thiopurine methyltransferase on mercaptopurine and thioguanine pharmacodynamics. Cancer Res 2007;67:4965-72; and Lennard L and Lilleyman JS. Individualizing therapy with 6-mercaptopurine and 6-thioguanine related to the thiopurine methyltransferase genetic polymorphism. Ther Drug Monit 1996;18:328-34). In addition, CPIC indicates that there is not a pharmacologically active secondary metabolite of thioguanine to undergo activation via TPMT (i.e., there are no methylthioinosine monophosphate or methylmercaptopurine nucleotides). As a result, patients receiving thioguanine are able to tolerate substantially higher 6-TGN concentrations than do those receiving mercaptopurine or azathioprine (Lennard and Lilleyman 1996). Finally, CPIC indicates that within each TPMT phenotypic group, the initial recommended relative dose decreases are similar for thioguanine, mercaptopurine, and azathioprine.

CPIC states that there is substantial evidence linking TPMT genotype with phenotypic variability. In addition, pre-emptive dose adjustments based on TPMT genotype have reduced thiopurine-induced adverse effects without compromising desired antitumor and immunosuppressive therapeutic effects in several clinical settings (Ford LT et al. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment: a pharmacogenomic test whose time has come. J Clin Pathol 2010;63:288-95; Relling MV et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 1999;91:2001-8; Schmiegelow K et al. Thiopurine methyltransferase activity is related to the risk of relapse of childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. Leukemia 2009;23:557-64; Schmiegelow K et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 2010;24:345-54; and Meggitt SJ et al. Azathioprine dosed by thiopurine methyltransferase activity for moderate-to-severe atopic eczema: a double-blind, randomised control-led trial. Lancet 2006;367:839-46).

Therapeutic recommendations for thioguanine are based on azathioprine/6-mercaptopurine. CPIC states that, if starting doses are already high (e.g., 75 mg/m^2 of 6-mercaptopurine), as is true in some ALL treatment regimens, lower than normal starting doses should be considered in TPMT IM (Stocco G et al. Genetic polymorphism of inosine triphosphate pyrophosphatase is a determinant of mercaptopurine metabolism and toxicity during treatment for acute lymphoblastic leukemia. Clin Pharmacol Ther 2009;85: 164-72; Lennard L et al. Individualizing therapy with 6-mercaptopurine and 6-thioguanine related to the thiopurine methyltransferase genetic polymorphism. Ther Drug Monit 1996;18:328-34; Schmiegelow K et al. Thiopurine methyltransferase activity is related to the risk of relapse of childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. Leukemia 2009;23:557-64; and Schmiegelow K et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 2010;24:345-54) and markedly reduced doses (10-fold reduction) should be used in TPMT PM (Evans WE et al. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphocytic leukemia. J Pediatr 1991;119:985-9). This approach has decreased the risk of acute toxicity without compromising relapse rate in ALL (Relling MV et al. Thiopurine methyltransferase in acute lymphoblastic leukemia. Blood 2006;107:843-4). Even at these markedly reduced dosages, erythrocyte 6-TGN concentrations in TPMT PM remain well above those tolerated and achieved by the majority of patients (who are TPMT NM (Ford LT et al. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment: a pharmacogenomic test whose time has come. J Clin Pathol 2010;63:288-95; and Evans WE et al. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphocytic leukemia. J Pediatr 1991;119:985-9).

CPIC indicates that in some non-malignant conditions, alternative agents may be chosen for IM or PM rather than reduced doses of thiopurines; if thiopurines are used, full starting doses are recommended for NM, reduced doses (30-80% of target dose) in IM (Meggitt SJ et al. Azathioprine dosed by thiopurine methyltransferase activity for moderate-to-severe atopic eczema: a double-blind, randomised controlled trial. Lancet 2006;367:839-46; and Coenen MJ et al. Identification of patients with variants in TPMT and dose reduction reduces hematologic events during thiopurine treatment of inflammatory bowel disease. Gastroenterology 2015;149:907-17), and substantially reduced doses (or use of an alternative agent) in PM (Ford LT et al. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment: a pharmacogenomic test whose time has come. J Clin Pathol 2010;63:288-95; and Sandborn WJ. Rational dosing of azathioprine and 6-mercaptopurine. Gut 2001;48: 591-2).

CPIC indicates that some of the clinical data upon which dosing recommendations are based rely on measures of TPMT phenotype rather than genotype; however, because TPMT genotype is strongly linked to TPMT phenotype (Schaeffeler E et al. Comprehensive analysis of thiopurine S-methyltransferase

phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenetics 2004;14:407-17; Yates CR et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med 1997;126:608-14; Liu 2017; and Tamm R et al. Polymorphic variation in TPMT is the principal determinant of TPMT phenotype: a meta-analysis of three genome-wide association studies. Clin Pharmacol Ther 2017;101:684-95), these recommendations apply regardless of the method used to assess TPMT status.

For thioguanine, CPIC classifies the recommendation for PM as strong (i.e. "the evidence is high quality and the desirable effects clearly outweigh the undesirable effects") and the recommendation for IM and possible IM as moderate (i.e. "There is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects").

The therapeutic recommendations for thioguanine are indicated below:

Dosing recommendations for thioguanine by TPMT phenotype		
Phenotype	Therapeutic recommendation	Classification of recommendation
IM (one no function allele: *2, *3A, *3B, *3C, *4, *11, *14, *15, *23, or *29) or possible IM (one allele with uncertain function (allele other than *1, *2, *3A, *3B, *3C, *4, *11, *14, *15, *23, or *29) and one no function allele)	Start with reduced doses (50-80% of normal dose) if normal starting dose[a] is ≥ 40-60 mg/m^2/day (e.g., 20-48 mg/m^2/day) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 2-4 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing thioguanine over other agents[b].	Moderate[c]
PM (two no function alleles: *2, *3A, *3B, *3C, *4, *11, *14, *15, *23, or *29)	Start with drastically reduced doses[d] (reduce daily dose[a] by 10-fold and dose thrice weekly instead of daily) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 4-6 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing thioguanine over other agents. For non-malignant conditions, consider alternative non-thiopurine immunosuppressant therapy.[e]	Strong[f]

[a] Normal starting doses vary by race/ethnicity and treatment regimens. If standard dose is below normal recommended dose, dose reduction might not be recommended for intermediate metabolisers.

[b] McBride 2000, and Ford LT and Berg JD. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment: a pharmacogenomic test whose time has come. J Clin Pathol 2010; 63:288-95.

[c] The classification moderate indicates that "there is a close or uncertain balance" as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.

[d] McBride 2000.

[e] Ford LT and Berg JD. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment: a pharmacogenomic test whose time has come. J Clin Pathol 2010;63:288-95.

[f] The classification strong indicates that the evidence is high quality and the desirable effects clearly outweigh the undesirable effects.

Recommendations for patients having also a NUDT15 variant

CPIC states that there have been reports of patients with intermediate metaboliser status for both TPMT and NUDT15 (i.e., compound intermediate metabolisers), and that there was a trend for a lower thiopurine tolerance in these individuals compared with intermediate metabolisers for only TPMT or NUDT15. However, CPIC indicates that the evidence for a different starting dose recommendation for the compound intermediate metabolisers remains limited.

The therapeutic recommendations for patients having also a NUDT15 variant are indicated below:

Dosing recommendations for thioguanine for patients with a genetically reduced activity for both TPMT and NUDT15		
TPMT phenotype	NUDT15 phenotype	Therapeutic recommendation

IM	IM	Consider dose reduction[a]. See TPMT IM and NUDT15 IM recommendation[b].
IM	PM	Dose reduction recommended[a]. See NUDT15 PM recommendation.
PM	IM	Dose reduction recommended[a]. See TPMT PM recommendation.
PM	PM	Dose reduction recommended[a]. See TPMT PM recommendation.

[a] Whether a dose reduction is recommended from the starting dose depends on the level of the standard starting dose; for example, if the standard starting dose of mercaptopurine is 75 mg/m^2/day or higher, then a lower starting dose may be considered in intermediate metabolisers and would be recommended in poor metabolisers, whereas if the starting dose is 50 mg/m^2/day or lower, a reduced starting dose may not be necessary in intermediate metabolisers.

[b] For patients who are intermediate metabolisers for both TPMT and NUDT15, further dose reduction might be needed compared with those who are only intermediate metabolisers with respect to one gene (TPMT or NUDT15).

As evidence linking TPMT genotype with thioguanine phenotype, CPIC mentions 11 articles. 4 of these articles were included in our risk analysis (Lennard 2015, Wray 2014, Lennard 2006, and McBride 2000). 6 articles were not included in our risk analysis because they concerned in vitro or preclinical (mouse) studies (Karim H et al. Differential role of thiopurine methyltransferase in the cytotoxic effects of 6-mercaptopurine and 6-thioguanine on human leukemia cells. Biochem Biophys Res Commun 2013;437:280-6; Hosni-Ahmed A et al. Thiopurine methyltransferase predicts the extent of cytotoxicity and DNA damage in astroglial cells after thioguanine exposure. PLoS One 2011;6:e29163; Hartford C et al. Differential effects of targeted disruption of thiopurine methyltransferase on mercaptopurine and thioguanine pharmacodynamics. Cancer Res 2007;67:4965-72; Krynetski E and Evans WE. Drug methylation in cancer therapy: lessons from the TPMT polymorphism. Oncogene 2003;22:7403-13; Hill DL et al. Inhibition of guanine metabolism of mammalian tumor cells by the carbocyclic analogue of adenosine. Mol Pharmacol 1971;7:375-80; and Moore EC and Le PG. The metabolism of 6-thioguanine in normal and neoplastic tissues. Cancer Res 1958;18:1075-83). 1 article was not included, because it studied the effect of TPMT enzyme activity, not TPMT genotype, on adverse effects and there was a later article of the same group focussing more on thioguanine (Stoneham S et al. Veno-occlusive disease in patients receiving thiopurines during maintenance therapy for childhood acute lymphoblastic leukaemia. Br J Haematol 2003;123:100-2). Our risk analysis includes 6 articles that were not included by CPIC. 2 of these articles were published after the last literature search performed by CPIC (van der Burg and Gerding 2018 and McAtee 2017). The other 4 articles were not (Mares 2009, Teml 2005, Herrlinger 2004, and Standen 2001).

CPIC indicates that the included in vitro studies provide a high level of evidence for thioguanine catabolism to methylthioguanine (Moore EC and Le PG. The metabolism of 6-thioguanine in normal and neoplastic tissues. Cancer Res 1958;18:1075-83), for heterologous expression of TPMT catabolizing mercaptopurine to methylmercaptopurine, thioguanine to methylthioguanine, and TIMP to methylTIMP (Krynetski E and Evans WE. Drug methylation in cancer therapy: lessons from the TPMT polymorphism. Oncogene 2003;22:7403-13, and Hill DL et al. Inhibition of guanine metabolism of mammalian tumor cells by the carbocyclic analogue of adenosine. Mol Pharmacol 1971;7:375-80), and for a higher sensitivity of TPMT knock-down cells to thioguanine compared to wild type (Karim H et al. Differential role of thiopurine methyltransferase in the cytotoxic effects of 6-mercaptopurine and 6-thioguanine on human leukemia cells. Biochem Biophys Res Commun 2013;437:280-6). One in vitro study provided a low level of evidence that TPMT deficiency could lead to chronic exposure to thioguanine and could be linked to development of brain cancer (astrocytomas) (Hosni-Ahmed A et al. Thiopurine methyltransferase predicts the extent of cytotoxicity and DNA damage in astroglial cells after thioguanine exposure. PLoS One 2011;6:e29163). CPIC indicates that the preclinical study combined with a preclinical study on 6-mercaptopurine provides a high level of evidence for TPMT knock-out mice having more morbidity and mortality but better ALL efficacy from thioguanine and mercaptopurine than wild type mice; heterozygotes were at intermediate risk (Hartford C et al. Differential effects of targeted disruption of thiopurine methyltransferase on mercaptopurine and thioguanine pharmacodynamics. Cancer Res 2007;67:4965-72, and an article on mercaptopurine). CPIC indicates that clinical studies provide a high level of evidence for TPMT PM having life-threatening toxicity (myelosuppression) from normal doses of mercaptopurine, thioguanine and azathioprine; toxicity can be minimized with substantially decreased doses (McBride 2000 and 14 mercaptopurine/azathioprine references). In addition, CPIC reports that clinical studies provide a moderate level of evidence that TPMT variant genotype is NOT associated with greater likelihood of event free survival, but studies that adjust dose based on TPMT status or tolerance may be unlikely to find such associations (Lennard 2015 and 5 references on mercaptopurine and/or azathioprine only). Finally, CPIC reports a weak level of evidence that TPMT activity is not associated with sinusoidal obstruction syndrome (Wray 2014, Lennard 2006, Stoneham S et al. Veno-occlusive disease in patients receiving thiopurines during maintenance therapy for childhood acute lymphoblastic leukaemia. Br J Haematol 2003;123:100-2, and a meta-analysis on mercaptopurine/azathioprine studying hepatotoxicity instead of sinusoidal obstruction syndrome), and that TPMT status is associated with development of secondary cancer (Len-

nard 2015 and 5 references on mercaptopurine and/or azathioprine only).
On 7-7-2023, there was not a more recent version of the recommendations present on the CPIC-site.

- Cost-effectiveness:
  - van der Wouden CH et al. Cost-effectiveness of pharmacogenomics-guided prescribing to prevent gene-drug-related deaths: a decision-analytic model. Front Pharmacol 2022;13:918493. PMID: 36120299.
    The costs per gene-drug-related death averted by genotype-guided treatment, was calculated for 148,128 Dutch patients starting one of seven drugs (azathioprine, 6-mercaptopurine, capecitabine, clopidogrel, systemic fluorouracil, irinotecan or tioguanine). The number of thioguanine initiators was 2,854. Genotype-guided treatment was according to the KNMP Pharmacogenetics Working Group recommendations. For PM, it was assumed that the recommendation for a dose decrease was followed, not the recommenddation for an alternative drug. For thioguanine, the costs per death averted by TPMT genotyping and TPMT-guided therapy was € 385,084. With a threshold of additional costs of € 20,000-60,000 per quality-adjusted life year (QALY) gained, this would be cost-effective if patients lived afterwards for 6-19 years with optimal quality of life.
    Testing of CYP2C19, DPYD, TPMT or UGT1A1 for start of clopidogrel, capecitabine, systemic fluorouracil, azathioprine, mercaptopurine, tioguanine or irinotecan was cost-effective (additional costs of € 51,000 per prevented gene-drug-related death).
    Costs were calculated over a period of 1 year and from a health care perspective. Only costs of genetic tests, health care professional interpretation, and drugs were included in the calculation. For the 2854 azathioprine initiators, TPMT-guided treatment was calculated to prevent 0.9 gene-drug-related deaths in the first year of treatment (decrease from 6.5 to 5.5 deaths) against additional costs of € 385,084 per prevented death. Costs for thioguanine were € 2.98 per 21 mg capsule, € 2.75 per 16 mg capsule and € 2.49 per 10 mg capsule, costs for pharmacist time were € 12.11 per 18 minutes, costs for physician time were € 4.28 per 6 minutes, and TPMT genotyping costs were € 132. The risk of gene-drug-related death was derived from literature review, the incidence of drug initiation from Dutch prescription data, and the predicted phenotype category frequencies from a Dutch sample of 1,023 individuals. For TPMT PM, it was assumed that the recommendation for a dose decrease was followed, not the recommendation for an alternative drug.

Date of literature search: 7 July 2023.

	Phenotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	PM	2F	Yes	Yes	25 September 2023
	IM	3E	Yes	Yes	

Mechanism:
Lower metabolic activity of TPMT leads to increased intracellular concentrations of thioguanine nucleotides, the active metabolites of thioguanine. This increases the risk of adverse events such as myelosuppression.
Thioguanine is a prodrug, which is converted into the active metabolites (thioguanine nucleotides) by the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT).
Two catabolic routes reduce thioguanine bioavailability for thioguanine nucleotide formation. Thiopurine methyltransferase (TPMT) catalyses S-methylation of thioguanine. Guanase converts thioguanine to the inactive metabolite 6-thioxanthine by deamination; 6-thioxanthine is subsequently converted to 6-thiouric acid by xanthine oxidase.

Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

Potentially beneficial	PGx testing for this gene-drug pair is potentially beneficial. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the DPWG recommends adhering to the gene-drug guideline	0-2 +
Beneficial	PGx testing for this gene-drug pair is beneficial. It is advised to consider genotyping the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3-5 +
Essential	PGx testing for this gene-drug pair is essential for drug safety or efficacy. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection	6-10 +

Table 2: Criteria on which the attribution of Clinical Implication Score is based

Clinical Implication Score Criteria	**Possible Score**	**Given Score**
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)		
• CTCAE Grade 3 or 4 (clinical effect score D or E)	+	
• CTCAE Grade 5 (clinical effect score F)	++	++
Level of evidence supporting the associated clinical effect grade ≥ 3		
• One study with level of evidence score ≥ 3	+	
• Two studies with level of evidence score ≥ 3	++	
• Three or more studies with level of evidence score ≥ 3	+++	+++
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3		
• 100 < NNG ≤ 1000	+	+
• 10 < NNG ≤ 100	++	
• NNG ≤ 10	+++	
PGx information in the Summary of Product Characteristics (SmPC)		
• At least one genotype/phenotype mentioned	+	+
OR		
• Recommendation to genotype	++	
OR		
• At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	++	
Total Score:	10+	7+
Corresponding Clinical Implication Score:		Essential