TPMT: thioguanine

ALL = acute lymphoblastic leukaemia, Clor = oral clearance, cytostat = cytostatic agent, EM = extensive metaboliser (normal TPMT enzyme activity: *1/*1), IM = intermediate metaboliser (reduced TPMT enzyme activity: *1/variant), imm sup = immunosuppressant, MR = metabolic ratio, 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

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 IM, especially for PM.

This is why a decision was made that this concerns a gene-drug interaction and that physicians must therefore make an active choice either to reduce the dose and/or to administer an alternative (yes/yes monitoring).

An overview of the clinical and kinetic effects per phenotype group is provided in the background information text of the relevant pharmacogenetic guideline on the KNMP Knowledge Database. You may also have access to this background text via your pharmacy or physician information system. Substantiation of the dose recommendation is provided below per phenotype group.

Justification of the dose recommendation per phenotype group

IM: A study including 40 IM patients showed that the median concentration of the active metabolite was 30% higher in EM patients, despite equal median doses. This is equivalent to a dose reduction to 77% to achieve the same median concentration of the active metabolite in IM patients as in EM 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 medicinal products (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.

PM: The literature describes evidence of dose adjustment in 2 PM patients (to 7.14% and 6.25% respectively; mean 6.7%). The levels found are consistent with the levels found for mercaptopurine/azathioprine (10%) considering that these medicinal products can be administered at relatively higher doses in patients with reduced TPMT activity, because these medicinal products are converted by TPMT to metabolites that contribute to the 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 choice of choosing an alternative is also included in the recommendation.

Source  Code  Effect  Comments
ref. 1 - cytostat  Lennard L et al.  Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: the influence of thiopurine methyltransfer- 3 426 children with acute lymphoblastic leukaemia were treated with thioguanine for 2 years. The initial dose was 40 mg/m² for EM and IM, and 4.0 mg/m² for PM. Thioguanine was administered in combination with methotrexate, vincristine and either dexamethasone or prednisone. Relevant co-medications were 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 EM (*1/*1)  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
IM: E
- 40x IM (1x *1/*2, 33x *1/*3A, 4x *1/*3C, 1x *1/*21, 1x *1/*34)
- 1x PM (*3A/*3A)

PM; A (2)

IM versus EM:
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 EM/IM) (S)
- 5-year EFS (event-free survival) with an event defined as time to relapse or death) increased by 10% for *1/*3A versus EM (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)
- 5-year EFS decreased by 34% in *1/*3C patients versus EM 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)

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 EM:
Thioguanine only:
- The eventual dose was 6.25% of the dose in EM 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 EM (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).

Median 6-TGN concentration versus EM:
IM: 130%
Dose versus EM:
PM: 6.25%

ref. 2 - cytostat
Wray L et al.
TPMT and MTHFR genotype is not associated with altered risk of thioguanine-related sinusoidal obstruction

3
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 EM, 54x IM+PM. (Patients in whom one of the two polymorphisms could not be identified were assumed to be wild-type.)

Authors’ conclusion: “TPMT and MTHFR C677T genotypes were not associated with sinusoidal obstruction syndrome risk.”

ref. 2, continuation

- *3B: 256x EM, 35x IM+PM (The genotype was unknown for 49 patients.)
- *3C: 302x EM, 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.

ref. 3 - cytostat

| IM: AA | 1492 children with ALL were randomised to maintenance therapy with either thioguanine at an initial dose of 40 mg/m²/day (n=748) or 6-mercaptopurine at an initial dose of 75 mg/m²/day (n=744). The thiopurine dose was titrated to toxicity guided by neutrophil and platelet counts. Co-medication: non-relevant cytostatic agents and steroids. Patients with thioguanine-related hepatic veno-occlusive disease 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/m²/day increased from median 1916 to 2034 pmol/8x10⁸ 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/m²/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.

Authors’ conclusion: “Thioguanine was associated with liver damage in 11% of children 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. 4 - cytostat
Standen GR et al. Heterozygosity for the thiopurine methyltransferase *3A allele in an acute non-lymphoblastic leukaemia

| IM+PM: AA | 2 | Patient with acute non-lymphoblastic leukaemia received thioguanine 100 mg/m² 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 <100x10⁹/L on Day 80 and the patient still required RBC transfusions.

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...
<table>
<thead>
<tr>
<th>Reference</th>
<th>IM: C</th>
<th>Patient with delayed marrow regeneration following H-DAT chemotherapy. Br J Haematol 2001;112:1089. ref. 4, continuation</th>
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<tr>
<td>ref. 5</td>
<td>IM: C</td>
<td>The patient was found to be an IM (*1/*3A). regimes that approach maximum haemopoietic tolerance.</td>
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**ref. 5 - cytostat**

McBride KL et al.

| ref. 5 | PM: F | An eight-year-old boy with ALL received consolidation therapy of thioguanine 50 mg/m²/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. |

**ref. 6 – imm supp**

Mares WG et al.

| ref. 6 | 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/8x10⁸ 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/8x10⁸ 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/8x10⁸ 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/8x10⁸ 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 EM: PM: 7.14% |

**ref. 7 – imm supp**

Temi A et al.
A prospective, open-label trial of 6-thioguanine in patients with ulcerative or  

| ref. 7 | PM: 3* | 16 patients with inflammatory bowel disease and intolerance or resistance to azathioprine/6-mercaptopurine (15x EM, 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 EM: up to 60 mg/day, 1 EM: up to 80 mg/day); adverse-event-Authors’ conclusion: “In the present study, TPMT did not help in explaining 6-TG-related side effects.” |

| IM: C(2) | 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 (EM) 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. |

* 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.

| IM: D(2) | 26 patients with Crohn’s disease (25x EM, 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 EM (abnormal liver tests that recovered without dose reduction and mild leukopenia) and the IM (signs of myelotoxicity: mild leukopenia, thrombocytopenia and anaemia). The two EM 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 pmol/8x10^8 RBC in a group of 9x EM and 1x IM). |

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 marrow depression.”

| 0 | PM: E | There are individuals with an inherited deficiency of the enzyme TPMT who may be unusually sensitive to the myelosuppressive effect of tioguanine and prone to developing rapid bone marrow depression following the initiation of treatment with tioguanine. This problem could be exacerbated by co-administration with drugs that inhibit TPMT, such as olsalazine, mesalazine or sulphasalazine. Some laboratories offer testing for TPMT deficiency, although these tests have not been shown to identify all patients at risk of severe toxicity. Therefore close monitoring of blood counts is still necessary. |

Risk group Use of TPMT inhibitors (mesalazine, olsalazine, sulphasalazine), use of inhibitors of de novo purine synthesis (methotrexate)

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 and Clin Pharmacol Ther 2013;93:324-5): recommended thioguanine doses are based on recommended mercaptopurine and azathioprine doses. The recommendations are: IM: the recommended initial dose is 50-70% of the EM dose; PM: the recommended initial dose is 10% of the EM daily dose at a reduced frequency of 3 times/week (instead of 7 times/week). The dose should be guided by...
myelosuppression and disease-specific guidelines, taking into consideration that steady state is delayed in PM patients (4-6 weeks) and IM patients (2-4 weeks) compared to EM patients (2 weeks).

Date of literature search: 31 March 2015.

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<td></td>
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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.