NUDT15: azathioprine/6-mercaptopurine 7035/7036

ALL = acute lymphoblastic leukaemia, CI = confidence interval, DNA-TG = DNA-incorporated 6-thioguanine-nucleotides, HR = hazard ratio, HWE = Hardy-Weinberg equilibrium, IBD = inflammatory bowel disease, IM = intermediate metaboliser (e.g. *1/*2, *1/*3) (reduced NUDT15 enzyme activity), NM = normal metaboliser (*1/*1) (normal NUDT15 enzyme activity), NS = not significant, NUDT15 = nudix hydroxylase 15 (enzyme involved in hydrolysis of diphosphate bonds and thus the conversion of 6-thio-deoxyguanosine triphosphate (6-thio-dGTP) into 6-deoxythioguanosine monophosphate (6-thio-dGMP)), OR = odds ratio, PM = poor metaboliser (e.g. *2/*2, *2/*3, *3/*3) (absent or strongly reduced NUDT15 enzyme activity), RBC = red blood cells, RR = risk ratio, S = significant, SNP = single nucleotide polymorphism, 6-TGN = 6-thioguanine nucleotides (the active metabolites of azathioprine/6-mercaptopurine), 6-thio-dGTP = 6-thio-deoxyguanosine triphosphate (the fully activated metabolite of azathioprine/6-mercaptopurine), TPMT = thiopurine S-methyltransferase (enzyme involved in formation of inactive thiopurine metabolites).

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:
NUDT15 reverses the final step in the formation of the active metabolite of 6-mercaptopurine and its precursor azathioprine. It converts 6-thiodeoxyguanosine triphosphate (6-thio-dGTP), which is incorporated into DNA, into 6-thiodeoxyguanosine monophosphate (6-thio-dGMP). For this reason, lower metabolic activity of NUDT15 leads to increased intracellular concentrations of the active metabolite 6-thio-dGTP. This increases the risk of adverse events such as myelosuppression.
All studies and meta-analyses included in the risk analysis confirmed that patients with genetically reduced NUDT-15 enzyme activity (intermediate metabolisers (IM) and poor metabolisers (PM)) have an increased risk for leukopenia and/or dose reduction due to adverse events like myelosuppression and infection (Yu 2023, Maeda 2022, Mao 2021, Tanaka 2021, Wang 2021, van Gennep 2019, Wang 2019, Park 2019, Liu 2018, Zhu 2018, Zhang 2018, Yi 2018, Kim 2018, Chao 2017, Yin 2017, Liang 2016, Zhu 2016, Moriyama 2016, Yang 2015, Yang 2014). In addition, two genotype-guided studies showed therapy adjustment for IM and PM to diminish the incidence of leukopenia in the whole group (Wang 2022 and Chao 2021). Wang 2022 showed in a group of 1013 patients with autoimmune disorders, among whom 248 IM+PM, that choosing an alternative for azathioprine in IM and PM decreased the incidence of leukopenia grade ≥ 2 with 95%, of leukopenia grade ≥ 3 with 94% and of leukopenia grade ≥ 4 with 100% compared to a historical control. Chao 2021 showed a decrease in leukopenia $<3.5x10^9$/L with 27%, decrease in leukopenia $<3.0x10^9$/L (grade ≥ 3) with 47%, and no decrease in effectiveness in 219 Crohn's disease patients , among whom 32 IM and 4 PM, treated with genotype-guided azathioprine therapy (half the normal starting dose for IM and an alternative for PM) compared to a control group of 204 patients receiving not-genotype guided azathioprine therapy. For this reason, the KNMP Pharmacogenetics Working Group decided that therapy adjustment is required for these gene-drug interactions (yes/yes-interactions).
Therapeutic recommendations
Because there is no assay for determining 6-thio-dGTP levels in patient cells, only maximum tolerated doses can be used to calculate dose adjustments. Ten included studies (Maeda 2022, Mao 2021, Tanaka 2021, Wang 2021, Park 2019, Yi 2017, Kim 2017, Chao 2017, Liang 2016 and Yang 2015) and one meta-analysis (Yin 2017) report tolerated doses. Calculations were only based on the ten studies because the meta-analysis also includes two of the studies. In addition, the meta-analysis does not specify the criteria for inclusion of studies for dose calculations and also included the studies of Moriyama 2016 and Yang 2014, which do not provide clear data on tolerated doses. Eight of the studies used for dose calculations concern acute lymphoblastic leukaemia (ALL) therapy (Mao 2021, Tanaka 2021, Wang 2021, Park 2019, Yi 2017, Kim 2017, Liang 2016, and Yang 2015). The other two studies concern inflammatory bowel disease therapy, but provide only data for IM (Maeda 2022 and Chao 2017).
PM: The weighted mean of the calculated dose adjustment for 57 PM from 8 ALL studies is a dose reduction to 16% (8-48%, median 16%). In ALL therapy, 6-mercaptopurine and methotrexate dose are usually lowered

alternately in case of leukopenia or other side effects. This indicates that using 16% of the normal 6-mercaptopurine dose in combination with normal methotrexate doses most probably will still result in leukopenia and the need for dose adjustment. For this reason, the KNMP Pharmacogenetics Working Group decided to recommend starting with the lower range of the observed tolerated doses, i.e. 8% of the normal dose, which was rounded off to 10% to make application in clinical practice more feasible. This would make the dosing recommendation the same as for TPMT PM. This corresponds to the similar effect of variants of these two genes on the tolerated thiopurine dose as observed in two studies comparing NUDT15 and TPMT directly (Liang 2016 and Yang 2015).
Because the dose adjustment could not be calculated, but had to be extrapolated, the KNMP Pharmacogenetics Working Group decided to recommend adjusting the dose in second instance and choosing another drug in first instance.

IM: The weighted mean of the calculated dose adjustment for 497 IM from 10 studies is a dose reduction to 71% (52-88%, median 73%). In ALL therapy, 6-mercaptopurine and methotrexate dose are usually lowered alternately in case of leukopenia or other side effects. In addition, in one of the inflammatory bowel disease studies, the tolerated dose was only calculated in patients tolerating azathioprine or 6-mercaptopurine for more than 6 months (60% of the NM, 47% of the IM and none of the PM) (Chao 2017). This suggests that in this study, the patients most sensitive to thiopurines were not included in the calculation. This indicates that using 70% of the normal 6-mercaptopurine dose either in combination with normal methotrexate doses or in the more sensitive halve of IM patients most probably will still result in leukopenia and the need for dose adjustment. For this reason, the KNMP Pharmacogenetics Working Group decided to recommend starting with the lower range of the observed tolerated doses, i.e. 52% of the normal dose, which was rounded off to 50% to make application in clinical practice more feasible. This would make the dosing recommendation the same as for TPMT IM. This corresponds to the similar effect of variants of these two genes on the tolerated thiopurine dose as observed in two studies comparing NUDT15 and TPMT directly (Liang 2016 and Yang 2015). 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 of the large overlap in the final dose range for NM and IM patients (final median dose approximately 80% and 60% of the starting dose for NM and IM, respectively (Yang 2015)), the KNMP Pharmacogenetics Working Group recommendation for these patients is to either start with 50% of the normal mercaptopurine dose or to start with the normal dose and reduce to 50% 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).

You can find a detailed overview of the observed kinetic and clinical effects in the background information text of the gene-drug interactions in the KNMP Kennisbank. You might also have access to this background text via your pharmacy or physician electronic decision support system.

Recommendation concerning pre-emptive genotyping, including justification of choices:

The KNMP Pharmacogenetics Working Group considers genotyping before starting azathioprine or 6-mercaptopurine to be essential for drug safety. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection.

The clinical implication of the gene-drug interaction scores 6 out of the maximum of 10 points for patients of European and African descend and 7 out of the maximum of 10 points for patients of Asian or (Latin-)American descend (with pre-emptive genotyping considered to be essential for scores ranging from 6 to 10 points):

The risk of serious life-threatening toxicity (code E corresponding to grade 4) is increased for patients with a genotype resulting in diminished NUDT15 enzyme activity (IM and PM). This results in 1 out of the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (1 point for CTCAE grade 3 or 4).

The increased risk for serious toxicity (code D-E corresponding to grade 3-4) has been shown in 13 studies (Mao 2021, Tanaka 2021, Wang 2021, Park 2019, Zhu 2018, Yi 2018, Kim 2018, Chao 2017, Liang 2016, Zhu 2016, Moriyama 2016, Yang 2015, and Yang 2014) and 2 meta-analyses (Zhang 2018 and Yin 2017). 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 was deduced from the increase in the percentage of patients with leukopenia for IM, which is the most prevalent variant genotype. For IM, an additional 23-77% of patients developed leukopenia compared to NM (Zhu 2018, Zhang 2018, and Chao 2017). The lower limit of 23% was used for the calculation. For Asians, Hispanics and Americans, the prevalence of IM has been reported to be 9.4-20.7%. This would amount to 2-5% of patients in these populations in which leukopenia is due to a NUDT15 variant and thus could have been prevented by reducing the risk to that in NM by lowering the dose, i.e. a number needed to genotype of 20-50. For Europeans and Africans, the prevalence of IM has been reported to be 0.6-1.5%. This would amount to 0.14-0.35%

of patients developing leukopenia due to a NUDT15 variant in these populations, i.e. a number needed to genotype of 300-700. The calculated number needed to genotype of 20-50 for Asians and (Latin-)Americans results in 2 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) to prevent one clinical effect grade ≥ 3 (2 points for 10 < NNG ≤ 100). The calculated number needed to genotype of 300-700 for Europeans and Africans results in 1 out of the maximum of 3 points (1 point for 100 < NNG ≤ 1000).
The Summaries of Product Characteristics (SmPCs) indicate that patients with a variant NUDT15 gene have an increased risk for severe toxicity of 6-mercaptopurine or azathioprine, such as early leukopenia and alopecia, at conventional doses of thiopurine therapy. 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).
In addition to the clinical implication score indicating pre-emptive genotyping to be essential in Asians and Latin-Americans, all 3 cost and cost-effectiveness analyses suggest that pre-emptive screening for NUDT15 in Asians is cost-saving (Wei 2022 and Zeng 2021) or cost-effective (Zarca 2020). Despite genotyping before starting azathioprine or 6-mercaptopurine also scoring as essential for drug safety in Whites and Africans, the only study analysing cost in Whites indicates that pre-emptive screening for NUDT15 in Whites is unlikely to be cost-effective (Zarca 2020). However, this study assumes that thiopurine-induced myelosuppression risk in IM is similar to that in NM, while studies and meta-analyses consistently showed a higher risk in IM.

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.

Source	Code	Effect	Comments
ref. 1 Yu N et al. Prevalence of NUDT-15 genetic variants and incidence of thiopurine-induced leukopenia in inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis 2023:jjad107. PMID: 37346013.	3	Meta-analysis of 11 studies investigating the effect of *3 on thiopurine-induced leukopenia in adult inflammatory bowel disease patients. The meta-analysis comparing *1/*3 and *1/*1 included 11 studies with a total of 3062 patients (2546 NM and 516 IM). The meta-analysis comparing *3/*3 and *1/*1 included 8 studies with a total of 2339 patients (2294 NM and 45 PM). Ten of the studies were cohort studies, one was a case-control study. Ten studies were performed in Asia, one in Australia. The thiopurine was not stated in one study and was azathioprine and/or 6-mercaptopurine in the other studies. All included studies met all 9 study quality checks on the Joanna Briggs Institute critical appraisal checklist. Leukopenia was defined as a white blood cell count below $3x10^9$/L in six studies, below $3.5x10^9$/L in four studies, and below $2.5x10^9$/L in one study. Two of the publications included in the meta-analysis are also included in this risk analysis separately (Chao 2017 and Zhu 2016). Seven of the publications included in the meta-analysis were also included the meta-analyses of Van Gennep 2019 and Liu 2018 (Sutiman 2018, Wang 2018, Chao 2017, Sato 2017, Asada 2016, Kakuta 2016, and Zhu 2016), six in the meta-analysis of Wang 2019 (Sutiman 2018, Wang 2018, Chao 2017, Sato 2017, Asada 2016, and Kakuta 2016),.and three in the meta-analyses of Zhang 2018 and Yin 2017 (Asada 2016, Kakuta 2016, and Zhu 2016). The meta-analyses were performed with a prospectively chosen random-effects model, and the study protocol was prospectively registered. The search and selection strategy was transparent and data extraction was standardised. Quality of the included studies was judged with a less common checklist designed for studies reporting prevalence data. Potential publication bias was assessed by funnel plot and	Authors' conclusion: 'NUDT15 variants are common and strongly predict thiopurine-induced leukopenia in IBD patients. Pre-treatment NUDT15 genotyping should be considered particularly in Asian populations to guide thiopurine dosing and prevent myelotoxicity.'

<table>
<tr>
<td>ref. 1, continuation</td>
<td>IM: C
PM: C</td>
<td>Egger’s test, but only for meta-analyses including more than 10 studies, so only for IM compared to NM and not for PM compared to NM.

Results:
<table>
<tr><td colspan="3">Leukopenia risk compared to NM:</td></tr>
<tr><td></td><td></td><td>incidence for NM</td></tr>
<tr><td>IM</td><td>RR = 4.12 (95% CI: 2.87-5.91) (S)</td><td>10.5%</td></tr>
<tr><td>PM</td><td>RR = 9.38 (95% CI: 5.17-17.01) (S)</td><td>9.9%</td></tr>
<tr><td colspan="3">The heterogeneity between the studies was high for both comparisons.</td></tr>
<tr><td colspan="3">There was no indication for publication bias for IM compared to NM.
Publication bias was not assessed for PM compared to NM.</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 2
Wang CW et al. Implementation of NUDT15 genotyping to prevent azathioprine-induced leukopenia for patients with autoimmune disorders in Chinese population. Clin Pharmacol Ther 2022;112:1079-87. PMID: 35869597.</td>
<td>3

Genotype-guided compared to non-genotype-guided treatment: AA#</td>
<td>1013 patients with autoimmune disorders received genotype-guided azathioprine treatment (azathioprine for NM and an alternative immunosuppressant for IM and PM) for 6 months. Genotyping was for gene variant 415C>T (*2 and *3). This is the most important gene variant in this Chinese patient group.
Comparison was with a historical cohort of 3244 patients receiving azathioprine treatment for autoimmune disorders for at least two weeks without genotyping.
Comedication with cyclosporine, methotrexate, and mycophenolate mofetil was excluded in both cohorts, as was chemotherapy or granulocyte-colony stimulating factor (G-CSF) prior to azathioprine. Sepsis or systemic lupus erythematosus (SLE) prior to azathioprine was excluded in the historical cohort.
Leukopenia grade ≥ 2 was defined as a white blood cell count below $3x10^9$/L, leukopenia grade ≥ 3 as a white blood cell count below $2x10^9$/L, and leukopenia grade ≥ 4 as a white blood cell count below $1x10^9$/L None of the patients on an alternative immunosuppressant developed leukopenia. Not all relevant comedications were excluded. In addition, a historical control group was used.

Genotyping:
- 765x NM
- 248x IM+PM

Results:
<table>
<tr><td colspan="3">% of azathioprine users with leukopenia for genotype-guided treatment compared to non-genotype-guided treatment (historical control):</td></tr>
<tr><td></td><td></td><td>value for the historical control</td></tr>
<tr><td>leukopenia grade ≥ 2</td><td>x 0.05 (S)</td><td>7.6%</td></tr>
<tr><td>leukopenia grade ≥ 3</td><td>x 0.06 (S)</td><td>5.2%</td></tr>
<tr><td>leukopenia grade ≥ 4</td><td>x 0 (S)</td><td>4.4%</td></tr>
<tr><td colspan="3">The positive predictive value (PPV) of gene variant 415C>T (*2 and *3) for development of azathioprine-induced leukopenia was 26.14% and the negative predic-</td></tr>
</table>
</td>
<td>Authors’ conclusion:
‘The genetic screening of NUDT15 R139C followed by use of alternative immunosuppressants in identified carriers effectively decreased the incidence of azathioprine leukopenia for patients with autoimmune disorders.’</td>
</tr>
</table>

<table>
<tr><td>ref. 2, continuation</td><td></td><td>tive value 99.22%.
The number needed to genotype to prevent one case of leukopenia grade ≥ 2 was 15.</td><td></td></tr>
<tr><td>ref. 3
Maeda T et al.
Long-term efficacy and tolerability of dose-adjusted thiopurine treatment in maintaining remission in inflammatory bowel disease patients with NUDT-15 heterozygosity.
Intest Res 2022;20:90-100.
PMID: 33472343.</td><td>3</td><td>142 patients with inflammatory bowel disease were treated with 6-mercaptopurine or azathioprine. Because all 4 PM discontinued treatment before maintenance therapy, this abstract only reports the data of the 138 non-PM patients. Median follow-up for these patients was 27 or 26 months. Doses are expressed in 6-mercaptopurine equivalents. The azathioprine doses were divided by 2.08 to obtain the 6-mercaptopurine equivalent doses. Thiopurines were administered at an initial 6-mercaptopurine equivalent dose of 0.2-0.6 mg/kg per day and screening for adverse events was conducted about 2 weeks after initiation. In case of adverse advents, the dose was decreased, thiopurine treatment was discontinued, or the thiopurine was changed, depending on the adverse events. If patients had no adverse events, the 6-mercaptopurine equivalent dose was increased as required to approximately 0.5 mg/kg per day.
The maintenance dose was defined as the final dose that patients could continue without adverse events. The serum levels of 6-TGN in maintenance therapy were assessed. Efficacy of thiopurine maintenance therapy in ulcerative colitis patients was defined as the cumulative clinical non-relapse rate (time from maintenance treatment start to clinical relapse) in patients who achieved remission withdrawing from steroids and without concomitant anti-TNF-α agents (n = 46). Efficacy of thiopurine maintenance therapy in Crohn's disease patients was defined as the cumulative surgery free rate (time from start of thiopurine/anti-TNF-α agent combination treatment to requirement of surgery or change in therapy) (n = 69).
Comedication affecting thiopurine metabolism was not excluded.

Genotyping:
All patients:
- 108x NM
- 30x IM
Not-discontinuing patients:
- 97x NM
- 27x IM

Results:
<table>
<tr><td colspan="3">Results for IM compared to NM:</td></tr>
<tr><td></td><td></td><td>value for NM</td></tr>
<tr><td>median 6-mercaptopurine equivalent maintenance (i.e. tolerated) dose (in mg/kg per day)</td><td>x 0.52 (S)</td><td>0.48</td></tr>
<tr><td>median 6-TGN level in maintenance therapy (in pmol/8x10^8 RBCs)</td><td>x 0.70 (S)</td><td>328</td></tr>
<tr><td>median duration of treatment (in months)</td><td>NS</td><td>27.0</td></tr>
<tr><td>% of patients discontinuing thiopurines</td><td>NS</td><td>10.2%</td></tr>
<tr><td>cumulative non-relapse rate for 60 months in ulcerative colitis patients</td><td>NS</td><td></td></tr>
<tr><td>cumulative surgery free</td><td>NS</td><td></td></tr>
</table></td><td>Authors' conclusion:
'Low-dose thiopurine treatment is an effective and acceptable treatment for patients with C/T genotype.'

Median maintenance (i.e. tolerated) 6-mercaptopurine equivalent dose compared to NM at a median starting dose of 0.28 or 0.26 mg/kg per day:
IM: 52%</td></tr>
</table>

<table>
<tr>
<td>ref. 3, continuation</td>
<td>IM: C</td>
<td>
<table>
<tr><td>rate without change of therapy for 60 months in Crohn’s disease patients</td><td></td><td></td></tr>
<tr><td>median 6-mercaptopurine equivalent initial dose (in mg/kg per day)</td><td>NS</td><td>0.28</td></tr>
<tr><td>white blood cell counts at 48, 60, 72, and 96 months after treatment start</td><td>decreased (S)</td><td></td></tr>
<tr><td>% of patients with leukopenia <3x10^9/L</td><td>x 7.4 (S)</td><td>0.9%</td></tr>
</table>
NOTE: Genotyping was for gene variant 415C>T (*2 and *3). This is the most important gene variant in this Japanese population.
</td>
<td></td>
</tr>
<tr>
<td>ref. 4
Chao K et al. Randomised clinical trial: dose optimising strategy by NUDT15 genotyping reduces leucopenia during thiopurine treatment of Crohn's disease. Aliment Pharmacol Ther 2021;54:1124-1133. PMID: 34563096.</td>
<td>4</td>
<td>
423 randomly assigned Crohn’s disease patients received either genotype-guided azathioprine treatment (n = 219) or the standard (not-genotype-guided) treatment (n = 204) for 36 weeks. Genotype-guided treatment consisted of the standard dose (2 mg/kg per day) for NM, 50% of the standard dose (1 mg/kg per day) for IM and an alternative drug for PM. If corticosteroids (0.75-1.0 mg/kg per day) were used for inducing clinical remission, they were tapered stepwise and stopped within 3 months. Gastroenterologists were allowed to change the azathioprine dose or stop treatment when a side effect occurred. Genotyping was for gene variant 415C>T (*2 and *3). This is the most important gene variant in this Chinese patient group.
The proportion of male patients was lower in the genotype-guided group than in the not-genotype-guided group.
Treatment efficacy was only determined in the 154 patients receiving azathioprine monotherapy with corticoids for clinical remission induction. Clinical remission was defined as Crohn's disease activity index < 150. Endoscopic response was defined as a decrease from baseline in the simple endoscopic score for Crohn's disease of more than 50%.
Comedication affecting thiopurine metabolism (allopurinol and 5-aminosalicylic acid) was excluded. In addition, multivariate analysis of leukopenia adjusted for concomitant use of steroids and concomitant use of infliximab, and treatment efficacy was only determined in patients receiving azathioprine monotherapy with corticoids for clinical remission induction.

Genotyping:
Genotype-guided group:
- 183x NM
- 32x IM
- 4x PM
Not-genotype-guided group:
- 154x NM
- 43x IM
- 7x PM

Results:
<table>
<tr><td colspan="3">Results for genotype-guided treatment compared to standard (non-genotype-guided) treatment:</td></tr>
<tr><td></td><td></td><td>value for standard treat-</td></tr>
</table>
</td>
<td>Authors’ conclusion:
‘Among Chinese patients with Crohn's disease, dose optimisation by NUDT15 C415T reduced the rate of thiopurine-induced leucopenia, without significant influence on efficacy. Using 50% dose reduction for heterozygotes, and alternative drugs for homozygotes, are practicable strategies.’</td>
</tr>
</table>

ref. 4, continuation	Genotype-guided compared to non-genotype-guided treatment: All: AA[#] NM: AA IM: AA[#] PM: AA[#]				ment	
		% of patients with leukopenia <3.5x10^9/L	all patients	RR = 0.73 (95% CI: 0.53-1.00) (S)	32.4%	
				Multivariate analysis showed pre-treatment genotyping to be an independent predictive factor for leukopenia: OR = 0.61 (95% CI: 0.41-0.95) (S)		
			NM patients	NS	20.1%	
			IM patients	RR = 0.48 (95% CI: 0.28-0.84) (S)	65.1%	
			PM patients	x 0 (S)	100%	
		% of patients with leukopenia <3x10^9/L		RR = 0.53 (95% CI: 0.30-0.96) (S)	13.7%	
		% of patients with leukopenia 3-3.5x10^9/L		NS	18.6%	
		% of patients with leukopenia <3.5x10^9/L in the first 8 weeks		RR = 0.55 (95% CI: 0.32-0.94) (S)	17.9%	
		% of patients with leukopenia <3x10^9/L after the first 8 weeks		NS	20.7%	
		% of patients discontinuing azathioprine due to leukopenia	all patients	x 0.58 (S)	21.9%	
			NM patients	NS		
			IM+PM patients	decreased (S)	> 70%	
		hepatotoxicity		NS	3.9%	
		pancreatitis		NS	2.5%	
		flu-like symptoms		NS	12.7%	
		rash		NS	4.9%	
		severe hair loss		NS	2.9%	
		gastric intolerance		NS	2.5%	
		C-reactive protein	0 months	NS		
			12 months	NS		
			36 months	NS		
				Also no difference for IM at all timepoints (NS).		
		erythrocyte sedimentation rate	0 months	NS		
			12 months	NS		
			36 months	NS		
				Also no difference for IM at all timepoints (NS).		
		Crohn's disease activity index	0 months	NS		
			12 months	NS		
			36 months	NS		
				Also no difference for IM at all timepoints (NS).		
		% of patients with steroid-free	all	NS	39.5%	
			NM	NS		
			IM	NS		

<table>
<tr><td>ref. 4, continuation</td><td></td><td>
<table>
<tr><td>clinical remission</td><td></td><td></td><td></td></tr>
<tr><td rowspan="3">% of patients with endoscopic response</td><td>all</td><td>NS</td><td>30.3%</td></tr>
<tr><td>NM</td><td>NS</td><td></td></tr>
<tr><td>IM</td><td>NS</td><td></td></tr>
<tr><td rowspan="3">6-TGN levels at 16 weeks (in pmol/8x108 RBCs)</td><td>all</td><td>NS</td><td>367.4</td></tr>
<tr><td>NM</td><td>NS</td><td></td></tr>
<tr><td>IM</td><td>NS</td><td></td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 5
Mao X et al.
Effects of TPMT, NUDT15, and ITPA genetic variants on 6-mercaptopurine toxicity for pediatric patients with acute lymphoblastic leukemia in Yunnan of China.
Front Pediatr 2021;9:719803.
PMID: 34660484.</td><td>3

IM+PM: D</td><td>149 children with acute lymphoblastic leukaemia received maintenance therapy with 6-mercaptopurine for 8-47 months (median 18 months). Maintenance therapy consisted of daily oral 6-mercaptopurine 50 mg/m^2, weekly oral methotrexate 20 mg/m^2, monthly intravenous vincristine 1.5 mg/m^2 (with a maximum of 2 mg), and monthly oral dexamethasone 6 mg/m^2 on day 1–5. Blood count was performed once or twice a week at the beginning of maintenance treatment and then at a 2-week interval to maintain white blood cell count of 2.0-3.0x10^9/L.
Leukopenia < 2.0x10^9/L only occurred in the first 12 weeks of maintenance therapy (after a median of 1 week, range 1-12 weeks). The tolerable dose was defined as the dose required to maintain a white blood cell count of 2.0-3.0x10^9/L, suggesting this dose to be determined after the last incidence of leukopenia < 2.0x10^9/L.
Leukopenia episodes during acute lymphoblastic leukaemia maintenance treatment generally do not lead to only decreases in the 6-mercaptopurine dose. Generally, 6-mercaptopurine and methotrexate doses are decreased alternately.

Genotyping:
- 109x NM
- 37x IM
- 3x PM

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td rowspan="2">tolerable dose</td><td>x 0.48 (S)</td><td>x 0.88 (trend: p = 0.074) (NS)</td><td rowspan="2">39.8 mg/m^2</td></tr>
<tr><td colspan="2">Note: the gene variant was associated with the tolerable dose (S).</td></tr>
<tr><td>% of patients with leukopenia <2x10^9/L</td><td colspan="2">x 1.8 (S)</td><td>33%</td></tr>
<tr><td>event free survival</td><td colspan="2">NS</td><td></td></tr>
</table>
NOTE: Genotyping was for gene variant 415C>T (*2 and *3). This is the most important gene variant in this Chinese population.</td><td>Authors' conclusion:
'NUDT15 c.415C>T (rs116855232) was an optimal predictor for 6-mercaptopurine toxicity and tolerable dose in pediatric ALL patients from Yunnan province, a multiethnic region in China, and would play an important role in precise therapy for ALL.'

Tolerated 6-mercaptopurine dose (i.e. resulting in leukopenia >2x10^9/L) compared to NM at a starting dose of 50 mg/m^2 per day:
IM: 88%
PM: 48%</td></tr>
<tr><td>ref. 6
Tanaka Y et al.
An international</td><td>3</td><td>37 paediatric PM with acute lymphoblastic leukaemia received maintenance therapy with 6-mercaptopurine and metho-</td><td>Authors' conclusion:
'Bi-allelic NUDT15</td></tr>
</table>

<table>
<tr>
<td>retrospective study for tolerability of 6-mercaptopurine on NUDT15 bi-allelic variants in children with acute lymphoblastic leukemia. Haematologica 2021;106:2026-9. PMID: 33504140.

ref. 6, continuation</td>
<td>PM: D
IM: D</td>
<td>trexate. Therapy typically started with 6-mercaptopurine 40 to 60 mg/m^2 per day and methotrexate 20 to 40 mg/m^2 per week. These doses were adjusted to maintain the target leukocyte count at 1.5-3.0x10^9/L. Because of earlier 6-mercaptopurine toxicity (and NUDT15 genotyping) in consolidation therapy, 73% of PM started maintenance therapy with 6-mercaptopurine < 30 mg/m^2 per day. In patients without a reduced starting dose, adjustment of the 6-mercaptopurine dose was difficult in most cases as the 6-mercaptopurine dose fluctuated dramatically and treatment interruption was common.
The tolerated dosages of 6-mercaptopurine and methotrexate were defined as the mean of the doses per day or per week, respectively, during the entire duration of maintenance therapy. This indicates that this included the doses (and treatment interruptions) in the first 200 days of maintenance treatment when most haematological toxicity was observed. The dose for the 37 PM was compared with the dose for 138 NM and 47 IM were derived from an earlier published study of the authors.
Most PM showed intolerance to 6-mercaptopurine. 86.5% required interruption of maintenance therapy, and the median duration of interruption for all patients was 47 days (range, 0-148 days). In patients with a 6-mercapopurine initial dose <10 mg/m^2, the total days of interruption during whole maintenance therapy was shorter than in patients with an initial dose of 10 mg/m^2 or more (S). 97% of PM developed neutropenia < 1x10^9/L (grade ≥ 3), 86.4% neutropenia < 0.5x10^9/L (grade ≥ 4), and 43.2% leukopenia ≤ 1x10^9/L (grade ≥ 4). The median observation times of neutropenia and leukopenia were 37 days (range, 9-139 days) and 33 days (range, 19-662 days), respectively, from start of maintenance therapy.
The median duration of follow-up was 1,398 days (range, 84-5,357 days) from the start of maintenance therapy. The four-year overall survival and event-free survival were 91% and 82%, respectively.
Methotrexate doses were also decreased. In addition, the maintenance dose was calculated over the whole maintenance period (including treatment interruptions), not only the final part when most patients actually tolerated the dose.

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td>maintenance dose (calculated over the whole period, including the part with haematological toxicity events)</td><td>x 0.12 (S)</td><td>x 0.81 (S)</td><td>41.7 mg/m^2</td></tr>
</table>

NOTE: Genotyping was by sequencing of NUDT15. Alleles *2, *3, *5, *6, and *7 were found.</td>
<td>variants conferred extreme intolerance to 6-mercaptopurine. Pre-emptive NUDT15 genotyping for all patients with ALL should be performed and dose modification in cases with bi-allelic variants must be considered. Precise upfront genotyping and a reduction of the 6-mercaptopurine dose to less to than 10 mg/m^2 is recommended to avoid the risk of severe complications and therapy interruption.’

Mean maintenance 6-mercaptopurine dose (i.e. titrated to obtain leukopenia 1.5-3.0 x10^9/L) compared to NM at a starting dose of typically 40-60 mg/m^2 per day:
IM: 81%
PM: 12%</td>
</tr>
<tr>
<td>ref. 7
Wang DS et al. Childhood acute lymphoblastic leukemia</td>
<td>3</td>
<td>216 children with acute lymphoblastic leukaemia received maintenance therapy with 6-mercaptopurine (initial dose 50-75, 60, 50, or 40 mg/m^2 per night depending on the protocol) and methotrexate (initial dose 40 mg/m^2 per week). Doses</td>
<td>Authors’ conclusion:
‘The major genetic determinant of mer-</td>
</tr>
</table>

<table>
<tr>
<td>mercaptopurine intolerance is associated with NUDT15 variants.
Pediatr Res 2021;89: 217-22.
PMID: 32221476.

ref. 7, continuation</td>
<td>IM: D
PM: D</td>
<td>were titrated to maintain a white blood cell count of 1.8-3x 10^9/L, absolute neutrophil count of 0.5-1.2x10^9/L, and platelet count ≥50x10^9/L. If the counts were low, 6-mercaptopurine was the first to be reduced in dose by a 25% decrement. If white blood cell or absolute neutrophil count failed to double at 1 week following dexamethasone pulse therapy, the 6-mercaptopurine and methotrexate doses were reduced to 50% of the initial dose given. If white blood cell or absolute neutrophil count remained the same or decreased, 6-mercaptopurine and methotrexate doses were halted because the participant would be at a high risk of infection. Blood counts after 3-4 days were used to determine whether the 6-mercaptopurine therapy could be resumed.
The tolerable dose was defined as the mean daily dose in the continuation phase.
Except for the first dose reduction, methotrexate doses were also reduced.

Genotyping:
- 158x NM
- 55x IM
- 3x PM

Results:
<table>
<tr><td colspan="3">Tolerable dose (mean daily dose in the continuation phase) compared to NM (36.8 mg/m^2):</td></tr>
<tr><td>IM</td><td>x 0.52 (NS)</td><td rowspan="2">S for PM versus IM versus NM</td></tr>
<tr><td>PM</td><td>x 0.12 (S)</td></tr>
<tr><td colspan="3">Multivariable linear regression showed only variants in NUDT15 to be associated with the mean daily 6-mercaptopurine dose (S).</td></tr>
</table>

NOTE: Genotyping was by sequencing of all three exons. In addition, sequencing of their parents' DNA was performed in patients with both gene variant 415C>T (exon 3) and gene variant 55_56insGAGTCG (exon 1) to establish whether these were on the same allele (genotype *1/*2) or on different alleles (genotype *3/*6). Variant alleles *2, *3, *5, *6, and *7 were found in this Japanese patient group.</td>
<td>captopurine intolerance was NUDT15 in Taiwanese patients.'

Tolerable 6-mercaptopurine dose (i.e. mean daily dose in the continuation phase) compared to NM at a starting dose of 40-75 mg/m^2 per day:
IM: 52%
PM: 12%</td>
</tr>
<tr>
<td>ref. 8
van Gennep S et al. Systematic review with meta-analysis: risk factors for thiopurine-induced leukopenia in IBD. Aliment Pharmacol Ther 2019;50:484-506.
PMID: 31342537.</td>
<td>3</td>
<td>Meta-analysis of 10 studies into azathioprine- or mercaptopurine-induced leukopenia and/or neutropenia in a total of 4994 patients with inflammatory bowel disease. The meta-analysis investigated the effect of gene variant 415C>T (*2 and *3). The meta-analysis comparing IM to NM included 9 studies with a total of 4914 patients. The meta-analysis comparing PM to NM included all 10 studies. All studies were performed in Asia. Six studies were cohort studies and four were case-control studies.
Of the 10 studies included in the meta-analysis, 1 scored the maximum of 9 points on the Newcastle-Ottawa quality assessment scale, 3 scored 8 points, 4 scored 7 points, 1 scored 6 points, and 1 scored 4 points. Studies with a score below 6 were considered to have high risk of bias. This single study was included both in the meta-analysis comparing IM to PM and the meta-analysis comparing PM to NM.
Leukopenia and/or neutropenia was defined as a white blood cell count below 3.0x10^9/L (7 studies) or 3.5x10^9/L (3 studies) and/or a neutrophil count below 1.5x10^9/L.
Three of the studies included in the meta-analysis are also</td>
<td>Authors' conclusion:
'TPMT and NUDT-15 variants predict thiopurine-induced leukopenia. Potential preventive measures to reduce the risk of thiopurine-induced leukopenia include pre-treatment TPMT and NUDT15 genotyping.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 8, continuation</td>
<td>IM: C
PM: C</td>
<td>included in this risk analysis separately (Chao 2017, Zhu 2016, and Wang 2014).
Nine of the studies included in the meta-analysis were also included in the meta-analysis of Liu 2018 (Sutiman 2018, Wang 2018, Chao 2017, Kim 2017, Sato 2017, Asada 2016, Kakuta 2016, Zhu 2016, and Yang 2014), eight in the meta-analysis of Wang 2019 (Sutiman 2018, Wang 2018, Chao 2017, Kim 2017, Sato 2017, Asada 2016, Kakuta 2016, and Yang 2014) and four in the meta-analyses of Zhang 2018 and Yin 2017 (Asada 2016, Kakuta 2016, Zhu 2016, and Yang 2014).
The meta-analyses were performed with a prospectively chosen random-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent and data extraction was standardised.
Potential publication bias was not assessed.

Results:
<table>
<tr><td colspan="2">% of patients with leukopenia and/or neutropenia compared to NM:</td></tr>
<tr><td>IM</td><td>OR = 5.9 (95% CI: 4.4-8.0) (S)</td></tr>
<tr><td>PM</td><td>OR = 69.9 (95% CI: 34.1-143.3) (S)</td></tr>
<tr><td colspan="2">Positive en negative predictive values for development of leukopenia and/or neutropenia were:
- 55% en 17% for IM
- 97% and 17% for PM</td></tr>
<tr><td colspan="2">Heterogeneity between the studies was significant and moderate for:
- PM compared to NM.
Heterogeneity between the studies was absent for:
- IM compared to NM.</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 9
Wang R et al. Association between the c.415C > T, c.52G > A, and 36_37insGGAGTC polymorphisms of NUDT 15 and thiopurine-induced leukopenia, thiopurine intolerance, and severe hair loss: an updated meta-analysis.
Drug Des Devel Ther 2019;13:2729-44.
PMID: 31496650.</td>
<td>3</td>
<td>Meta-analysis of 11 studies investigating the effect of gene variant 415C>T (*2 and *3) on azathioprine- or mercaptopurine-induced leukopenia. The meta-analysis comparing IM and NM included all 11 studies and a total of 2717 patients (2195 NM and 522 IM). The meta-analysis comparing PM and NM included 10 studies with a total of 2191 patients (2131 NM and 60 PM). All studies were performed in Asia. Eight studies were performed in inflammatory bowel disease patients, of which 1 also included paediatric patients, One study was performed in patients with inflammatory bowel disease or auto-immune hepatitis. One study, also including paediatric patients, was performed in patients with several diseases, including myasthenia gravis, chronic inflammatory demyelinating polyneuropathy, neuromyelitis optica, and vasculitis. One study was performed in paediatric acute lymphoblastic leukaemia patients.
Of the 11 studies included in the meta-analysis, 2 scored the maximum of 9 points on the Newcastle-Ottawa quality assessment scale, 3 scored 8 points, and 6 scored 7 points. The definition of leukopenia in the included studies was not stated, but it was indicated that some studies analysed leukopenia grade 3-4 separately, indicating that also leukopenia < grade 3 was included.
Two of the studies included in the meta-analysis are also included in this risk analysis separately (Chao 2017 and Yang 2014).
Nine of the studies included in the meta-analysis were also</td>
<td>Authors' conclusion:
'NUDT 15 c.415C > T polymorphism could increase the risk of leukopenia, early/late leukopenia, leukopenia (grade 3-4), and severe hair loss. Meanwhile, c.52G > A and c.36_37ins GGAGTC mutations also probably increase the risk of leukopenia. Preemptive tests for NUDT 15 polymorphisms are highly recommended to individualize the treatment of thiopurine for a better outcome with less toxicity.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 9, continuation</td>
<td>IM: C
PM: C</td>
<td>included in the meta-analysis of Liu 2018 (Sutiman 2018, Wang 2018, Chao 2017, Kim 2017, Sato 2017, Shah 2017, Asada 2016, Kakuta 2016, and Yang 2014), five in the meta-analysis of Zhang 2018 (Shah 2017, Asada 2016, Kakuta 2016, Tanaka 2015, and Yang 2014) and four in the meta-analysis of Yin 2017 (Asada 2016, Kakuta 2016, Tanaka 2015, and Yang 2014).
Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of absent or low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and data extraction was standardised.
Potential publication bias was not assessed.

Results:
<table>
<tr><td colspan="3">Leukopenia risk compared to NM:</td></tr>
<tr><td></td><td></td><td>incidence for NM</td></tr>
<tr><td>IM</td><td>OR = 6.41 (95% CI: 5.19-7.94) (S)</td><td>19%</td></tr>
<tr><td>PM</td><td>OR = 45.60 (95% CI: 18.84-110.37) (S)</td><td>19%</td></tr>
<tr><td colspan="3">59% of IM and 97% of PM developed leukopenia.</td></tr>
<tr><td colspan="3">The heterogeneity between the studies was not significant for both comparisons and absent for the comparison of PM to NM.</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 10
Park Y et al.
Star allele-based haplotyping versus gene-wise variant burden scoring for predicting 6-mercaptopurine intolerance in pediatric acute lymphoblastic leukemia patients.
Front Pharmacol 2019;10:654.
PMID: 31244663.</td>
<td>3

IM: E
PM: E</td>
<td>244 children with acute lymphoblastic leukaemia received maintenance therapy with 6-mercaptopurine. The treatment protocol was the same for all patients. Doses were titrated to maintain an absolute neutrophil count of 0.5-1.5x10^9/L.
The maximum tolerated 6-mercaptopurine dose was defined as the dose at the last maintenance cycle for each patient.
The mean maximum tolerated 6-mercaptopurine dose for the whole patient group was 65.16 mg/m^2.
Neutropenia episodes during acute lymphoblastic leukaemia maintenance treatment generally do not lead to only decreases in the 6-mercaptopurine dose. Generally, 6-mercaptopurine and methotrexate doses are decreased alternately.

Genotyping:
- 195x NM
- 48x IM
- 1x PM

Results:
<table>
<tr><td colspan="2">Maximum tolerated dose intensity (the dose at the last maintenance cycle as percentage of the planned dose) compared to NM (67.608%):</td></tr>
<tr><td>IM</td><td>x 0.83 (S)</td></tr>
<tr><td>PM</td><td>x 0.08 (trend, p = 0.09) (NS)</td></tr>
<tr><td colspan="2">NOTE: The difference between PM and IM was significant (S).</td></tr>
</table>

NOTE: Genotyping was by whole exome sequencing. Gene variants 415C>T (*2 and *3), 55_56insGAGTCG (*2 and *6), 416G>A (*4), 52G>A (*5), and 50delGAGTCG (*9) were found in this South Korean patient group. Alleles based on</td>
<td>Authors' conclusion:
'Last-cycle dose intensity percent showed significant differences among NUDT15 poor (PM, n = 1), intermediate (IM, n = 48), and normal (NM, n = 195) metabolizers.'

Maximum tolerated 6-mercaptopurine dose (i.e. (the dose at the last maintenance cycle) compared to NM:
IM: 83%
PM: 8%</td>
</tr>
</table>

<table>
<tr><td>ref. 10, continuation</td><td></td><td>the first 4 gene variants (i.e. *2-*6) were inferred by PHASE 2.1.1 software.</td><td></td></tr>
<tr><td>ref. 11
Liu Y et al. Associations between the NUDT15 R139C polymorphism and susceptibility to thiopurine-induced leukopenia in Asians: a meta-analysis. Onco Targets Ther 2018;11:8309-17. PMID: 30538500.
.</td><td>3

IM: C</td><td>Meta-analysis of 14 studies, including 2566 NM, 625 IM, and 68 PM, investigating the effect of gene variant 415C>T (*2 and *3) on azathioprine- or mercaptopurine-induced leukopenia and/or neutropenia. In 12 studies, including 1939 NM, 451 IM, and 55 PM, genotype distribution in the patients not developing leukopenia and/or neutropenia was in Hardy-Weinberg equilibrium (HWE). Eight studies, including 2070 NM, 521 IM, and 45 PM, were performed in inflammatory bowel disease (IBD) patients. Four studies were performed in acute lymphoblastoid leukaemia patients, one in patients with neurological diseases, and one in patients with inflammatory bowel disease or auto-immune hepatitis. All studies were performed in Asia.
Of the 14 studies included in the meta-analysis, 6 scored the maximum of 9 points on the Newcastle-Ottawa quality assessment scale, 3 scored 8 points, 2 scored 7 points, and 3 scored 6 points.
The definition of leukopenia was whole blood cell count < $3.0x10^9$/L in 7 non-acute lymphoblastoid leukaemia studies, < $3.5\ x10^9$/L in the other 3 non-acute lymphoblastoid leukaemia studies, and < $2.0\ x10^9$/L in the acute lymphoblastoid leukaemia studies. One acute lymphoblastoid leukaemia study investigated only neutropenia (defined as neutropenia < $0.5x10^9$/L), and one acute lymphoblastoid leukaemia study and one inflammatory bowel disease study investigated also neutropenia (defined as < $1.0\ x10^9$/L and < $1.5x10^9$/L, respectively).
Four of the studies included in the meta-analysis are also included in this risk analysis separately (Zhu 2018, Chao 2017, Zhu 2016 and Yang 2014).
Five of the studies included in the meta-analysis were also included in the meta-analyses of Zhang 2018 (Shah 2017, Asada 2016, Chienthong 2016, Kakuta 2016, and Yang 2014) and Yin 2017 (Asada 2016, Chienthong 2016, Kakuta 2016, Zhu 2016, and Yang 2014).
Meta-analyses were performed with a random-effects model in case of moderate to high heterogeneity between the studies and with a fixed-effects model in case of absent or low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and data extraction was standardised.
Potential publication bias was assessed by funnel plot and Egger's test, but only for IM+PM compared to NM, not for IM compared to NM and PM compared to NM, and only for all 14 studies, not for only the Hardy-Weinberg equilibrium studies and only the inflammatory bowel disease studies.

Results:
<table>
<tr><td colspan="4">Leukopenia and/or neutropenia risk compared to NM:</td></tr>
<tr><td></td><td>studies</td><td></td><td>incidence for NM</td></tr>
<tr><td rowspan="2">IM</td><td>all</td><td>OR = 7.60 (95% CI: 4.97-11.61) (S)</td><td>19%</td></tr>
<tr><td>HWE</td><td>OR = 7.85 (95% CI: 4.87-12.65) (S)</td><td>19%</td></tr>
</table>
</td><td>Authors' conclusion:
'This meta-analysis verified the strong association between the NUDT-15 R139C polymorphism and thiopurine-induced leukopenia (both early and late leukopenia) in an Asian population with IBD, ALL, and other diseases. NUDT15 R139C genotyping should be prioritized to predict leukopenia among Asians.'</td></tr>
</table>

<table>
<tr><td>ref. 11, continuation</td><td>PM: C</td><td>
<table>
<tr><td></td><td>IBD</td><td>OR = 6.55 (95% CI: 4.42-9.70) (S)</td><td>19%</td></tr>
<tr><td rowspan="3">PM</td><td>all</td><td>OR = 38.5 (95% CI: 17.8-83.2) (S)</td><td>19%</td></tr>
<tr><td>HWE</td><td>OR = 40.8 (95% CI: 17.0-97.9) (S)</td><td>19%</td></tr>
<tr><td>IBD</td><td>OR = 51.2 (95% CI: 17.4-150.5) (S)</td><td>19%</td></tr>
<tr><td colspan="4">60% of IM and 96% of PM in all 14 studies developed leukopenia and/or neutropenia.</td></tr>
<tr><td colspan="4">The heterogeneity between the studies was significant for IM compared to NM and not-significant for PM compared to NM.</td></tr>
<tr><td colspan="4">There were no indications for publication bias for all studies for IM+PM compared to NM.
Possible indication bias was not assessed for IM compared to NM and PM compared to NM, and not for the 12 Hardy-Weinberg equilibrium studies and 8 inflammatory bowel disease studies.</td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 12
Zhu Y et al.
Combination of common and novel rare NUDT15 variants improves predictive sensitivity of thiopurine-induced leukopenia in children with acute lymphoblastic leukemia.
Haematologica 2018;103:e293-e295.
PubMed PMID: 29519865.</td><td>4

IM+PM: D</td><td>188 children with acute lymphoblastic leukaemia received chemotherapy with 6-mercaptopurine according to standard protocol during 1 year. 6-mercaptopurine was used in the remission induction phase (standard dosage of 60 mg/m^2 in the last two weeks), the consolidation phase (25 mg/m^2), and the maintenance phase (50 mg/m^2).
48 patients (25.5%) experienced 6-mercaptopurine-induced leukopenia, requiring reduction of 6-mercapopurine dose, interruptions of therapy, and/or prescription of human granulocyte colony-stimulating factor (G-CSF). 37 patients (19.7%) experienced 6-mercaptopurine-induced hepatotoxicity, which was defined as a more than 5-fold increase of aspartate transaminase and/or alanine transaminase levels after introducing 6-mercaptopurine.

Genotyping:
*2 and *3: - 151x NM - 37x IM+PM (31x IM, 6 PM)
*2 and *6: - 172x NM - 16x IM+PM
*5: - 185x NM - 3x IM
In addition, one carrier of each of the following 3 gene variants was found:
- rs149436418 (C>G) (Phe52Leu)
- rs761191455 (->G) (Glu115Gly and frameshift resulting in a truncated protein without enzymatic domain)
- rs751671087 (A>G) (Gly161Arg)

Results:
<table>
<tr><td colspan="4">Results compared to no gene variant:</td></tr>
<tr><td></td><td>gene variant(s)</td><td>gene variant carriers</td><td>value for no gene variant</td></tr>
<tr><td rowspan="4">% of patients with leukopenia</td><td rowspan="4">*2 and *3</td><td>x 9.0 (S)</td><td rowspan="4">10%</td></tr>
<tr><td>The sensitivity of *2 and *3 to predict leukopenia was 68.8% and the specificity 97.1%.</td></tr>
<tr><td>All 6 homozygous carriers (PM) had leukopenia (sensitivity and specificity of 100%).</td></tr>
<tr><td>A homozygous carrier who was also heterozy-</td></tr>
</table>
</td><td>Authors' conclusion:
'We found the significantly association between the reported/novel NUDT15 and TPMT SNPs with thiopurine-induced leukopenia but not hepatotoxicity. Patients with NUDT15$^{risk/risk}$TPMT$^{wt/risk}$ genotype will suffer more severe leukopenia, and should be adjusted into a much lower initial dosage of 6-mercaptopurine in clinical level. Therefore, detection of all potential functional variants in these two genes is strongly recommended in individualized usage of 6-mercaptopurine in ALL treatment.'</td></tr>
</table>

ref. 12, continuation				gous for TPMT *3 experienced a much earlier and more severe myelosuppression than the rest of the patients (leukopenia grade 3, neutropenia and thrombocytopenia grade 4 after 2 weeks of consolidation therapy (6-mercaptopurine dose 25 mg/m^2)). The final 6-mercaptopurine dose in the maintenance phase was 2.5% of the normal dose (i.e. 2.5 mg/m^2 every two days).	
			*2 and *6	x 3.2 (S)	22%
				NS after correction for the 415C>T polymorphism (identifying *2 and *3) in multivariate analysis.	
			*5	NS	25%
			Phe52Leu	NS	25%
				The only carrier developed leukopenia and the final 6-mercaptopurine dose was 80% of the normal dose.	
			Glu115Gly + frameshift	NS	25%
				The only carrier developed leukopenia and the final 6-mercaptopurine dose was 67% of the normal dose.	
			Gly161Arg	NS	25%
				The only carrier developed leukopenia and the final 6-mercaptopurine dose was 50% of the normal dose. However, this patient was also heterozygous for *3, so it is not clear whether Gly161Arg conferred additional leukopenia risk. However, a protein stability assay suggested this protein to have a lower stability than the wild type protein.	
		% of patients with hepatotoxicity	*2 and *3	NS	21%
			*2 and *6	NS	19%
			*5	NS	19%
			Phe52Leu	NS	20%
			Glu115Gly + frameshift	NS	20%
			Gly161Arg	NS	20%

<table>
<tr><td>ref. 12, continuation</td><td></td><td>NOTE: Genotyping was by sequencing of NUDT15.</td><td></td></tr>
<tr><td>ref. 13
Zhang AL et al. Association of NUDT15 c.415C>T allele and thiopurine-induced leukocytopenia in Asians: a systematic review and meta-analysis. Ir J Med Sci 2018;187:145-153. PubMed PMID: 28470355.</td><td>4

IM: D
PM: D</td><td>Meta-analysis of 7 studies investigating the effect of *2 and *3 on thiopurine-induced leukopenia (1138 patients in total; 827 NM, 277 IM and 34 PM). Six of the studies were cohort studies, one was a case-control study. Two studies with a total of 174 patients were acute lymphoblastic leukaemia studies, the other were inflammatory bowel disease studies. All patients were Asian. The thiopurine was 6-mercaptopurine or azathioprine.
Of the 7 studies included in the meta-analysis, 4 scored the maximum of 9 points on the Newcastle-Ottawa quality assessment scale, 1 scored 7 points, and 2 scored 6 points. Two of the publications included in the meta-analysis are also included in this risk analysis separately (Zhu 2016 and Yang 2014).
Six of the publications included in the meta-analysis were also included in the meta-analysis of Yin 2017 (Asada 2016, Chiengthong 2016, Kakuta 2016, Zhu 2016, Tanaka 2015, and Yang 2014). The meta-analysis of Yin 2017 included also the non-Asian patients in Chiengthong 2016 (181 Guatemalan patients).
For the meta-analysis a random-effects model was used in case of significant heterogeneity between the studies and a fixed-effects model otherwise. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and data extraction was standardised.
Potential publication bias was assessed by funnel plot and Egger’s test.

Results:
<table><tr><td colspan="2">Percentage of patients with leukopenia compared to NM (13.4%):</td></tr><tr><td>IM</td><td>RR = 3.41 (95% CI: 2.44-4.77) (S)</td></tr><tr><td>PM</td><td>RR = 6.54 (95% CI: 3.34-12.82) (S)</td></tr><tr><td colspan="2">The heterogeneity between the studies was high.</td></tr><tr><td colspan="2">There was no indication for publication bias.</td></tr><tr><td colspan="2">The calculated RRs were similar after removing each study one at a time from the meta-analysis.</td></tr></table></td><td>Authors’ conclusion:
‘The results of this meta-analysis confirm that NUDT15 c.415C>T may be an important predictor of thiopurine-induced leukocytopenia in Asians. Genotype targeting of NUDT15 c.415 C>T before initiating thiopurine treatment may be useful to limit leukocytopenia.’</td></tr>
<tr><td>ref. 14
Yi ES et al. NUDT15 variants cause hematopoietic toxicity with low 6-TGN levels in children with acute lymphoblastic leukemia. Cancer Res Treat 2018;50:872-82. PubMed PMID: 28903549.</td><td>4</td><td>182 children with acute lymphoblastic leukaemia without TPMT variants received maintenance therapy with 6-mercaptopurine during 1 year. Vincristine, prednisolone, methotrexate, cytarabine and hydrocortisone were also included in the maintenance therapy. The initial 6-mercaptopurine dose was 50 mg/m^2 daily. White blood cell counts and red blood cell levels of 6-TGN were determined at day 14 after start of maintenance therapy and at day 14 after dose alteration, until the white blood cell count and 6-TGN levels were within the target range. 6-Mercaptopurine and methotrexate doses were altered at the discretion of paediatric oncologists based on the complete blood count and 6-TGN levels. When the white blood cell count was out of target range (1.5-3.0x 10^9/L), the 6-mercaptopurine dose was adjusted if 6-TGN levels were out of therapeutic range (235-450 pmol/8×10^8 red blood cells (RBC)), or the methotrexate dose was altered if 6-TGN levels were within the therapeutic range. Treatment was interrupted if patients developed significant hematopoietic toxicity (absolute neutrophil count < 0.5x10^9/L or platelet</td><td>Authors’ conclusion:
‘NUDT15 variants cause hematopoietic toxicity with low 6-TGN levels. NUDT15 genotyping should be conducted before administering thiopurine, and dose adjustments require caution regardless of 6-TGN levels.’</td></tr>
</table>

<table>
<tr>
<td>ref. 14, continuation</td>
<td>IM: D

PM: E</td>
<td>count < 50x10^9/L) or a serious infectious event.
Leukopenia was defined as a white blood cell count below 2x10^9/L. 6-TGN levels were determined approximately 5 times in 182 patients (67% of NM, 52% of IM and all PM). 6-TGN levels of patients with a stable 6-mercaptopurine dose for at least 14 days were analysed.

Genotyping:
- 131x NM
- 46x IM (17x *1/*2, 25x *1/*3, 3x *1/*5, 1x *1/*6)
- 5x PM (3x *2/*3, 1 x *2/*4, 1x *3/*3)

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td>average 6-mercaptopurine dose 3-12 months after therapy start</td><td>x 0.24 (S)</td><td>x 0.78 (S)</td><td>31.1 mg/m^2 per day</td></tr>
<tr><td>number of days with therapy interruption</td><td>x 11 (S)</td><td>x 2.0 (S)</td><td>15.5 days</td></tr>
<tr><td rowspan="2">% of patients with febrile neutropenia</td><td>x 5.5 (S)</td><td>x 1.04 (NS)</td><td rowspan="2">14.6%</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td>lowest white blood cell count</td><td>x 0.46 (S)</td><td>x 0.82 (S)</td><td>1.36x 10^9/L</td></tr>
<tr><td rowspan="2">lowest absolute neutrophil count</td><td>x 0.092 (S)</td><td>x 0.68 (NS) (trend for a decrease (p = 0.061)</td><td rowspan="2">0.434x 10^9/L</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td rowspan="2">lowest haemoglobin levels</td><td>x 0.73 (S)</td><td>x 0.97 (NS)</td><td rowspan="2">10.1x 10^9/L</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td>lowest platelet count</td><td>x 0.066 (S)</td><td>x 0.84 (S)</td><td>137x 10^9/L</td></tr>
<tr><td>number of days with leukopenia</td><td>x 2.2 (S)</td><td>x 1.6 (S)</td><td>59 days</td></tr>
<tr><td rowspan="3">median 6-TGN level</td><td>x 0.20</td><td>x 0.75</td><td rowspan="3">383 pmol/8 x10^8 RBC</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td colspan="2">The 6-TGN levels in PM were below the alleged level associated with a higher risk of leukopenia (450 pmol/8x10^8 RBC). In 26 results from the PM group, 10 (38.5%) were performed while the patient had grade ≥ 3 leukopenia or thrombocytopenia (platelets < 50x10^9/L).</td></tr>
<tr><td rowspan="2">6-TGN level/6-mercaptopurine dose ratio</td><td>x 0.30 (S)</td><td>NS</td><td rowspan="2">14.7</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
</table>
</td>
<td>Average dose 3-12 months after therapy start compared to NM at a starting dose of 50 mg/m^2 per day:
IM: 78%
PM: 24%</td>
</tr>
</table>

<table>
<tr><td>ref. 14, continuation</td><td></td><td>NOTE: Genotyping was by sequencing of exons 1 and 3 (identifying *2-*6). These are the most important gene variants in this Korean population.</td><td></td></tr>
<tr><td>ref. 15
Kim H et al.
APEX1 polymorphism and mercaptopurine-related early onset neutropenia in pediatric acute lymphoblastic leukemia.
Cancer Res Treat 2018;50:823-34.
PubMed PMID: 28882023.</td><td>4

IM: D

PM: E</td><td>183 children with acute lymphoblastic leukaemia received maintenance therapy with 6-mercaptopurine. Median follow-up was 74.2 months (26.6-235.7 months). Children’s Cancer Group protocols were used for the maintenance therapy with the initial 6-mercaptopurine dose modified from 75 to 50 mg/m^2 per day. Doses of 6-mercaptopurine during maintenance therapy were adjusted to maintain a white blood cell count of 2.0-3.5x10^9/L with an absolute neutrophil count over 500/µl, and hepatotoxicity related dose modifications were performed at the discretion of the treating physician. Methotrexate doses were also adjusted during therapy. In acute lymphoblastic leukaemia maintenance therapy, 6-mercaptopurine and methotrexate doses are usually alternatively reduced in response to observed drug toxicity. Doses of drugs were not prospectively adjusted based on patient genotypes.
Maintenance therapies used in patients with standard-risk acute lymphoblastic leukaemia (31%) and in patients with high-risk acute lymphoblastic leukaemia (69%) were different modifications of the same protocol.
Neutropenia was defined as an absolute neutrophil count below 0.5x10^9/L (neutropenia grade 4). The dose in the final 12-weeks maintenance cycle was considered to be the maximum tolerated dose for each patient. This dose was available for 171 patients (82% of NM, 97% of IM and all PM).

Genotyping:
- 142x NM
- 37x IM
- 4x PM

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td rowspan="3">maximum tolerated 6-mercaptopurine dose</td><td>x 0.19</td><td>x 0.65</td><td rowspan="3">26.9 mg/m^2 per day</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td colspan="2">Note: The methotrexate dose reduction was also higher for PM versus IM versus NM (S), indicating that the maximum tolerated 6-mercaptopurine dose for IM and PM might be even smaller if the methotrexate dose would be adjusted in the same extent in PM, IM and NM.</td></tr>
<tr><td rowspan="3">% of patients with neutropenia grade 4 in the first 720 days</td><td>x 1.5 (S)</td><td>x 1.1 (NS)</td><td rowspan="3">65.0%</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td colspan="2">Multivariate analysis showed PM to be an independent risk factor for neutropenia grade</td></tr>
</table></td><td>Authors’ conclusion:
‘APEX1 and NUDT-15 both contribute to cell protection from DNA damage or misincorporation, so alleles that impair the function of either gene may affect 6-mercaptopurine sensitivities, thereby inducing 6-mercaptopurine-related neutropenia.’

Maximum tolerated 6-mercaptopurine dose compared to NM at a starting dose of 50 mg/m^2 per day:
IM: 65%
PM: 19%</td></tr>
</table>

<table>
<tr>
<td>ref. 15, continuation</td>
<td></td>
<td>
<table>
<tr><td></td><td>4 (HR = 11.4 (95% CI: 3.4-39.0) (S), but IM not (NS).</td><td></td></tr>
</table>

NOTE: In 40 patients who tolerated less than 25% of the planned 6-mercaptopurine dose, a 9655 bp stretch of the NUDT15 gene was sequenced. Subsequent genotyping was for the 415C>T polymorphism (*2 and *3). These are the most important gene variants in this Korean population.
</td>
<td></td>
</tr>
<tr>
<td>ref. 16
Chao K et al.
Combined detection of NUDT15 variants could highly predict thiopurine-induced leukopenia in Chinese patients with inflammatory bowel disease: a multicenter analysis.
Inflamm Bowel Dis 2017;23:1592-9.
PubMed PMID: 28570428.</td>
<td>4</td>
<td>
732 patients with inflammatory bowel disease were treated with azathioprine (target maintenance dose 2 mg/kg daily) (n = 692) or 6-mercaptopurine (target maintenance dose 1 mg/kg daily) (n = 40) for a median duration of 34.5 weeks (0.9-475.4 weeks). Dose adjustment of thiopurines was according to white blood cell count and other adverse events.
The median dose was 1.5 mg/kg per day. 24.3% of patients developed leukopenia. Leukopenia was defined as a white blood cell count below 3.5x10^{9}/L (grade ≥ 1). Early, middle and late leukopenia were defined as happening in the first 8 weeks, in week 8-24 and after more than 24 weeks, respectively. Complete blood counts and other regular laboratory measurements regarding adverse events and efficacy were assessed once a week in the first 2 months on thiopurines, and twice a month thereafter.
The average dose of thiopurines was calculated from the final dose of the patients who could continue thiopurines for more than 6 months (n = 405; 60% of the NM, 47% of the IM and none of the PM).
Co-medication was not excluded, but co-medication did not affect the prevalence of leukopenia.

Genotyping:
- 524x NM
- 192x IM (57x *1/*2, 102x *1/*3, 13x *1/*5, 20x *1/*6)
- 16x PM (6x *2/*3, 5x *3/*3, 1x *2/*6, 4x *3/*5)

Results:
<table>
<tr><td colspan="5">Results compared to no gene variant:</td></tr>
<tr><td></td><td>gene variant(s)</td><td>homozygous</td><td>heterozygous</td><td>value for no gene variant</td></tr>
<tr><td rowspan="4">% of patients with leukopenia</td><td rowspan="4">all</td><td>x 6.6</td><td>x 2.8</td><td rowspan="4">15.1%</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td colspan="2">The sensitivity of all gene variants for predicting leukopenia was 55.4%, with a specificity of 80.2%. The positive and negative predictive values were 47.1% and 84.9%.</td></tr>
<tr><td colspan="2"></td></tr>
<tr><td rowspan="4"></td><td rowspan="4">*2 and *3</td><td>x 6.2</td><td>x 2.9</td><td rowspan="4">16.2%</td></tr>
<tr><td colspan="2">S for homozygous versus heterozygous versus (no *2 and *3)</td></tr>
<tr><td colspan="2">OR = 4.45 (95% CI: 3.23-6.12) (S)</td></tr>
<tr><td colspan="2">Multivariate analysis sho-</td></tr>
</table>
</td>
<td>
Authors' conclusion:
'We confirmed that NUDT15 c.415C>T, c.36_37insGGAGTC, and c.52G>A variants were risk factors for thiopurine-induced leukopenia. Combined detection of the 3 variants could increase the predictive sensitivity of thiopurine-induced leukopenia and help to distinguish early leukopenia in heterozygote of c.415C>T in Chinese patients with IBD. Treatment monitoring by NUDT15 variants may be promising in individualized therapy.'
</td>
</tr>
</table>

ref. 16, continuation				wed the 415C>T polymorphism (identifying *2 and *3) to be an independent risk factor for leukopenia: OR = 4.54 (95% CI: 3.02-6.80) (S).			
				The sensitivity of *2 and *3 for predicting leukopenia was 49.2%, with a specificity of 84.1%.			
			*2 and *6	x 4.8	x 2.4	20.8%	
				S for homozygous versus heterozygous versus (no *2 and *6)			
				OR = 3.52 (95% CI: 2.26-5.49) (S)			
				Multivariate analysis showed a trend for the 36_37insGGAGTC polymorphism (identifying *2 and *6) to be an independent risk factor for leukopenia (p = 0.067) (NS). The 36_37insGGAGTC polymorphism was associated with leukopenia in both patients without *2 and *3 (n = 557; OR = 2.95 (95% CI: 1.14-7.90) (S)) and in patients without *5 (n = 715; OR = 3.96 (95% CI: 2.49-6.30) (S)).			
			*5	-	x 2.0 (S)	23.6%	
				OR = 2.83 (95% CI: 1.08-7.39) (S)			
				Multivariate analysis showed *5 to be an independent risk factor for leukopenia: OR = 4.63 (95% CI: 1.65-13.00) (S).			
	PM: E IM: E	% of leukopenia being grade 3-4	*2 and *3	x 11	x 2.6	5.6%	
				S for homozygous versus heterozygous versus (no *2 and *3)			
			*2 and *6	x 0	x 1.4	11.9%	
				S for homozygous versus heterozygous versus (no *2 and *6)			
			*5	-	x 3.2 (S)	11.8%	
		% of leukopenia being grade 2	*2 and *3	x 1.0	x 1.2	35.6%	
				S for (*2 and/or *3) versus (no *2 and *3)			
			*2 and *6	x 0	x 1.3	37.0%	
				S for (*2 and/or *6) versus (no *2 and *6)			
			*5	-	NS	39.6%	
		% of leukopenia being	*2 and *3	x 0	x 0.71	58.9%	
				S for (*2 and/or *3) versus (no *2 and *3)			
			*2 and	x 2.0	x 0.72	51.1%	

<table>
<tr>
<td>ref. 16, continuation</td>
<td></td>
<td>
<table>
<tr><td rowspan="2">grade 1</td><td>*6</td><td colspan="2">S for (*2 and/or *6) versus (no *2 and *6)</td><td></td></tr>
<tr><td>*5</td><td>-</td><td>NS</td><td>48.5%</td></tr>
<tr><td rowspan="3">% of leuko-penia being early (week 0-8)</td><td rowspan="2">*2 and *3</td><td>x 3.3</td><td>x 1.4</td><td rowspan="2">30.0%</td></tr>
<tr><td colspan="2">S for homozygous versus heterozygous versus (no *2 and *3)</td></tr>
<tr><td rowspan="2">*2 and *6</td><td>x 0</td><td>x 1.1</td><td rowspan="2">38.5%</td></tr>
<tr><td colspan="2">S for (*2 and/or *6) versus (no *2 and *6)</td></tr>
<tr><td>*5</td><td>-</td><td>x 2.0 (S)</td><td>37.9%</td></tr>
<tr><td rowspan="3">% of leuko-penia being middle (week 8-24)</td><td rowspan="2">*2 and *3</td><td>x 0</td><td>x 1.4</td><td rowspan="2">22.2%</td></tr>
<tr><td colspan="2">S for (*2 and/or *3) versus (no *2 and *3)</td></tr>
<tr><td rowspan="2">*2 and *6</td><td>x 0</td><td>x 1.0</td><td rowspan="2">24.4%</td></tr>
<tr><td colspan="2">S for (*2 and/or *6) versus (no *2 and *6)</td></tr>
<tr><td>*5</td><td>-</td><td>NS</td><td>24.3%</td></tr>
<tr><td rowspan="3">% of leuko-penia being late (> 24 weeks)</td><td rowspan="2">*2 and *3</td><td>x 0</td><td>x 0.58</td><td rowspan="2">47.8%</td></tr>
<tr><td colspan="2">S for (*2 and/or *3) versus (no *2 and *3)</td></tr>
<tr><td rowspan="2">*2 and *6</td><td>x 2.7</td><td>x 0.85</td><td rowspan="2">37.0%</td></tr>
<tr><td colspan="2">S for (*2 and/or *6) versus (no *2 and *6)</td></tr>
<tr><td>*5</td><td>-</td><td>NS</td><td>37.9%</td></tr>
<tr><td rowspan="4">median daily dose after more than 6 months</td><td>all</td><td>-</td><td>x 0.67 (S)</td><td>1.6 mg/kg</td></tr>
<tr><td>*2 and *3</td><td>-</td><td>x 0.65 (S)</td><td>1.6 mg/kg</td></tr>
<tr><td>*2 and *6</td><td>-</td><td>x 0.76 (NS)</td><td>1.5 mg/kg</td></tr>
<tr><td>*5</td><td>-</td><td>x 0.81 (NS)</td><td>1.5 mg/kg</td></tr>
</table>

NOTE: Genotyping was for the 415C>T polymorphism (*2 and *3), the 36_37insGGAGTC polymorphism (*2 and *6) and the 52G>A polymorphism (*5). These are the most important gene variants in this Chinese population.
</td>
<td>Median tolerated dose compared to NM at a starting dose of azathioprine 2 mg/kg per day:
IM: 67%</td>
</tr>
<tr>
<td>ref. 17
Yin D et al.
Impact of NUDT15 polymorphisms on thiopurines-induced myelotoxicity and thiopurines tolerance dose.
Oncotarget 2017;8:13575-85.
PubMed PMID: 28088792.</td>
<td>4</td>
<td>Meta-analyses of 7 studies investigating the effect of *2 and *3 on thiopurine-induced leukopenia (1752 patients in total) and of 13 studies investigating the effect of *2 and *3 on the tolerated thiopurine dose (2745 patients in total). The majority of patients was Asian. The thiopurine was 6-mercaptopurine or azathioprine.
The leukopenia meta-analysis included 3 acute lymphoblastic leukaemia publications with in total 225 patients (179 NM, 38 IM and 8 PM) and 4 inflammatory bowel disease publications with in total 1527 patients (1218 NM, 284 IM and 25 PM).
The tolerated dose meta-analysis included 9 acute lymphoblastic leukaemia cohorts from 5 publications with in total 1402 patients (1228 NM, 165 IM and 9 PM) and 4 inflammatory bowel disease publications with in total 1343 patients (1082 NM, 239 IM and 22 PM).
The quality of the included studies was judged based on the risk of bias in 4 parameters: phenotype definition, genotyping, population stratification, and selective reporting. Of the 7 included studies on thiopurine-induced leukopenia, 2 had a low risk of bias in all 4 parameters, 2 had a possible risk of</td>
<td>Authors' conclusion:
'Genetic polymorphisms in NUDT15 are strongly associated with adverse drug reaction of thiopurines, although more evidences are needed to determine values of all functional NUDT-15 polymorphisms for clinical regimen, rs116855232 should be considered as a highly credible pharmacogenetic indicator for thiopurines using espcially is Asians.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 17, continuation</td>
<td>PM: D
IM: D</td>
<td>bias in selective reporting, 1 had a possible risk of bias in the phenotype definition, 1 had a possible risk of bias in population stratification, and 1 had a possible risk of bias in the phenotype definition and an unknown risk of bias in genotyping. Of the 9 included studies on tolerated thiopurine dose, 3 had a low risk of bias in all 4 parameters, 4 had a possible risk of bias in selective reporting, 1 had a possible risk of bias in population stratification, and 1 had a possible risk of bias in the phenotype definition and an unknown risk of bias in genotyping.
Two of the publications included in the leukopenia meta-analysis (Zhu 2016 and Yang 2014) and four of the publications included in the tolerated dose meta-analysis (Moriyama 2016, Liang 2015, Yang 2015 and Yang 2014) are also included in this risk analysis separately.
6-mercaptopurine dose was converted to azathioprine equivalent dose using a conversion factor of 2.08, and Meeh-Rubner formula was used to unify the units into mg/m^2.
For the meta-analyses a random-effects model was used in case of heterogeneity between the studies. Otherwise, a fixed-effects model was used. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and data extraction was standardised.
Potential publication bias was assessed by funnel plot and Egger’s test.

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td>% of patients with leukopenia</td><td>OR = 18.10 (95% CI: 6.34-51.68) (S)</td><td>OR = 7.60 (95% CI: 5.77-10.03) (S)</td><td>24.5%</td></tr>
<tr><td></td><td colspan="2">The presence of *2 and/or *3 had a sensitivity of 43.2% and specificity of 91.7% for all leukopenia events, while the specificity reached 84.6% for early leukopenia events.</td><td></td></tr>
<tr><td>tolerated dose</td><td colspan="2">x 0.72 (95% CI: 0.66-0.79) (S)</td><td></td></tr>
<tr><td></td><td colspan="2">The dose reduction was similar in ALL and inflammatory bowel disease studies: dose reduction to 71% and 75% of the dose for NM respectively.</td><td></td></tr>
<tr><td></td><td colspan="2">The dose reduction in 23 PM was a dose reduction to 43% of the dose for NM and the dose reduction in 386 IM a dose reduction to 78% of the dose for NM (significance not determined).</td><td></td></tr>
<tr><td colspan="4">There was no heterogeneity between the studies in the leukopenia meta-analysis.
The heterogeneity between the studies was high in the tolerated dose meta-analysis, also the heterogeneity between the ALL studies and the heterogeneity between the inflammatory bowel disease studies.</td></tr>
</table>
</td>
<td>(Tolerated dose compared to NM:
IM: 78%
PM: 43%)</td>
</tr>
</table>

<table>
<tr><td>ref. 17, continuation</td><td></td><td>For both meta-analyses, there were no indications for publication bias.
The calculated ORs were similar after removing each study one at a time from the meta-analyses.</td><td></td></tr>
<tr><td>ref. 18
Liang DC et al.
NUDT15 gene polymorphism related to mercaptopurine intolerance in Taiwan Chinese children with acute lymphoblastic leukemia.
Pharmacogenomics J 2016;16:536-9.
PubMed PMID: 26503813.</td><td>4

PM: D
IM: D</td><td>310 children with standard- or high-risk acute lymphoblastic leukaemia received maintenance therapy with 6-mercaptopurine. Taiwan Pediatric Oncology Group protocols were used for the maintenance therapy. The initial 6-mercaptopurine dose was 60 mg/m^2 per day. Doses were adjusted to maintain a white blood cell count of 1.8-3.0x10^9/L with an absolute neutrophil count of 0.5-1.2x10^9/L and platelet count ≥ 50x10^9/L. If counts were low, 6-mercaptopurine was the first drug to be reduced in dose by 25% decrements. When 6-mercaptopurine dose was reduced by 50%, then methotrexate would start to be reduced in dose.
The maximum tolerated dose was defined as the maximum dose that the patient could tolerate for longer than 3 months till the end of continuation therapy.
The duration of event-free survival was defined as the time from the start of chemotherapy to any type of treatment failure (defined as relapse, death from any cause, development of second malignant neoplasm) or the day of last follow-up.

Genotyping:
- 238x NM
- 70x IM
- 2x PM

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td rowspan="2">maximum tolerated 6-mercaptopurine dose</td><td>x 0.21</td><td>x 0.70</td><td rowspan="2">44.1 mg/m^2 per day</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td>% of patients with 5-year event-free survival</td><td>-</td><td>NS</td><td>87.6%</td></tr>
<tr><td>% of patients with relapse</td><td>NS</td><td>NS</td><td>10.1%</td></tr>
<tr><td>% of patients with toxic death</td><td>NS</td><td>NS</td><td>0.8%</td></tr>
</table>

NOTE: For the 12 TPMT IM, the maximum tolerated 6-mercaptopurine dose was 0.71x the value for NUDT15 NM (S), indicating the effect of the TPMT variant to be similar to that of the NUDT15 variant.

NOTE: Genotyping was for the 415C>T polymorphism (*2 and *3). These are the most important gene variants in this Taiwanese population.</td><td>Authors' conclusion:
'The high frequency of risk variant for NUDT15, but not the very low frequency of risk variant for TPMT, was closely associated with the intolerance to mercaptopurine in children with ALL in Taiwan, contrast to that of European descent. In regard to NUDT15 polymorphism, the maximal tolerable daily doses of mercaptopurine in homozygotes, heterozygotes and wild-type groups were 9.4 mg m^{-2}, 30.7 mg m^{-2} and 44.1 mg m^{-2}, respectively. The outcomes did not differ significantly among the different genotypes.'

Maximum tolerated 6-mercaptopurine dose compared to NM at a starting dose of 60 mg/m^2 per day:
IM: 70%
PM: 21%</td></tr>
<tr><td>ref. 19
Zhu X et al.
NUDT15 polymorphisms are better than thiopurine S-methyltransferase as predictor of risk for thiopurine-induced leukopenia in Chi-</td><td>4</td><td>253 patients with Crohn's disease were treated with azathioprine or 6-mercaptopurine for a median duration of 38.0 weeks (1-192 weeks). In the first 2 weeks, azathioprine dose was 1 mg/kg daily and 6-mercaptopurine dose 0.5 mg/kg daily. If patients had no adverse reactions, the drug dose was increased to 2 mg/kg daily for azathioprine and 1 mg/kg daily for 6-mercaptopurine without alteration in the following weeks. The dose was reduced in case of leukopenia (white blood cell count < 3.5x10^9/L), neutropenia (neutrophil count</td><td>Authors' conclusion:
'In Chinese patients, it is strongly recommended to detect NUDT15 genotype rather than TPMT before initiating thiopurine</td></tr>
</table>

<table>
<tr>
<td>nese patients with Crohn's disease. Aliment Pharmacol Ther 2016;44:967-75. PubMed PMID: 27604507.

ref. 19, continuation</td>
<td>PM: E
IM: D</td>
<td>< 1.5x10^9/L), severe hair loss (objective hair loss, that may cause patients to wear wigs, with a recovery time of a few months), or hepatotoxicity (aspartate transaminase or alanine transaminase more than two times the normal upper limit or alkaline phosphatase more than three times the normal upper limit). If any laboratory abnormality did not subside, the treatment was discontinued. Decisions about discontinuation or dose adjustment in patients who experienced other adverse events were taken by the responsible physician on a case-by-case basis.
Complete blood counts were determined once a week in the first month, then twice a month.
25.7% of patients developed leukopenia. 9.2% of patients with leukopenia also had severe hair loss and 33.8% had neutropenia during the follow-up visit.
Azathioprine/6-mercaptopurine metabolites in erythrocytes were measured in 154 patients after at least 4 weeks of administration of a stable dose or at the time of leukopenia. Co-medication with ciclosporin, methotrexate, and drugs potentially interfering with azathioprine or 6-mercaptopurine metabolism, including allopurinol, 5-aminosalicylates and diuretics, were excluded. There were no significant differences in co-medication between patients with or without leukopenia.

Genotyping:
- 196x NM
- 53x IM
- 4x PM

Results:
<table>
<tr><td colspan="4">Results compared to NM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td>% of patients with leukopenia</td><td>x 7.8</td><td>x 5.3</td><td>12.8%</td></tr>
<tr><td></td><td colspan="2">S for PM versus IM versus NM</td><td></td></tr>
<tr><td></td><td colspan="2">PM had leukopenia grade 3 or 4.</td><td></td></tr>
<tr><td>% of patients with early leukopenia (week 0-8)</td><td>x 24</td><td>x 6.9</td><td>4.1%</td></tr>
<tr><td></td><td colspan="2">S for PM versus IM versus NM</td><td></td></tr>
<tr><td>% of patients with middle leukopenia (week 8-24)</td><td>-</td><td>x 9.7 (S)</td><td>3.1%</td></tr>
<tr><td>% of patients with late leukopenia (> 24 weeks)</td><td>-</td><td>x 1.7 (S)</td><td>5.6%</td></tr>
<tr><td>% of patients with neutropenia</td><td colspan="2">x 11 (S for IM+PM compared to NM)</td><td>2.6%</td></tr>
<tr><td>correlation of 6-TGN concentrations with leukopenia</td><td colspan="2">no
The mean 6-TGN concentration in IM+PM with leukopenia was within the proposed therapeutic range (235–400 pmol/8x 10^8 red blood cells), whereas it was above the proposed therapeutic range in NM</td><td>yes</td></tr>
</table>
</td>
<td>drugs. 6TGN concentration should be routinely monitored in Crohn’s disease patients with NUDT15 wild type. As for CT genotype, starting at low dose and careful monitoring for leukopenia and 6TGN levels is recommended.’</td>
</tr>
</table>

<table>
<tr><td>ref. 19, continuation</td><td></td><td>
<table>
<tr><td></td><td>with leukopenia.</td><td></td></tr>
</table>

NOTE: Genotyping was for the 415C>T polymorphism (*2 and *3). These are the most important gene variants in this Chinese population.</td><td></td></tr>
<tr><td>ref. 20
Moriyama T et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity.
Nat Genet 2016;48:367-73.
PubMed PMID: 26878724.</td><td>4

PM: D
IM: D</td><td>270 children with acute lymphoblastic leukaemia and without TPMT variants (159 Guatemalan, 79 Singaporean and 32 Japanese) received maintenance therapy with 6-mercaptopurine. The planned 6-mercaptopurine dose was 50-75 mg/m^2 per day for the Guatemalan children, 50 mg/m^2 per day for the Singaporean children with standard- or intermediate-risk ALL, 75 mg/m^2 per day for the Singaporean children with high-risk ALL and 50 mg/m^2 per day for the Japanese children. Target white blood cell counts were 1.5-3.0x10^9/L for the Guatemalan children, 2.0-4.0x10^9/L for the Singaporean children and 2.0-3.0x10^9/L for the Guatemalan children. 6-mercaptopurine dose was adjusted during therapy to avoid host toxicities (myelosuppression and infections).
The maximum tolerated 6-mercaptopurine dose was defined as the average over at least 14 daily doses after at least 9 weeks of maintenance therapy.
The data of all 3 populations together were determined by meta-analysis using a random-effects model.
Levels of DNA-TG (DNA-incorporated thioguaninenucleotides) in white blood cells were determined in 32 Singaporean children and all 32 Japanese children and corrected for the 6-mercaptopurine dose 14 days prior to sampling. Correlations were investigated with linear regression analysis. So, correlations were corrected for confounders.

Genotyping:
- 221x NM
- 45x IM (*1/*2, *1/*3, *1/*4, *1/*5)
- 4x PM (*2/*3, *3/*3, *3/*5)

Results:
<table>
<tr><td colspan="5">Results compared to NM:</td></tr>
<tr><td></td><td>country</td><td>PM</td><td>IM</td><td>value for NM</td></tr>
<tr><td rowspan="8">median maximum tolerated 6-mercaptopurine dose</td><td rowspan="2">all</td><td colspan="2">decrease for PM versus IM versus NM (S)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">There was no significant heterogeneity between the three patient groups.</td></tr>
<tr><td rowspan="2">Guatemala</td><td></td><td>decrease (S)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">decrease for PM versus IM versus NM (S)</td></tr>
<tr><td rowspan="2">Singapore</td><td></td><td>decrease (S)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">decrease for PM versus IM versus NM (S)</td></tr>
<tr><td rowspan="2">Japan</td><td></td><td>decrease (S)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">decrease for PM versus IM versus NM (S)</td></tr>
<tr><td rowspan="2">median dose-corrected DNA-</td><td rowspan="2">Singapore + Japan</td><td colspan="2">increase for PM versus IM versus NM (S)</td><td rowspan="2"></td></tr>
<tr><td colspan="2">There was no significant heterogeneity between the two patient groups.</td></tr>
</table>
</td><td>Authors' conclusion:
'Loss-of-function NUDT15 diplotypes were consistently associated with thiopurine intolerance across three cohorts. …… Taken together, our results indicate that a comprehensive pharmacogenetic model integrating NUDT15 variants may inform personalized thiopurine therapy.'</td></tr>
</table>

<table>
<tr>
<td>ref. 20, continuation</td>
<td></td>
<td>
<table>
<tr><td>TG level</td><td></td><td colspan="2">There were no significant differences between genotypes within IM (*1/*2, *1/*3, *1/*5) and between genotypes within PM (*2/*3, *3/*3, *3/*5).</td><td></td></tr>
<tr><td rowspan="2">Singapore</td><td rowspan="2">x 3.3</td><td>x 1.5 (NS)</td><td rowspan="2">6.0 fmol/µg DNA/ mg</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td rowspan="2">Japan</td><td rowspan="2">x 3.4</td><td>x 1.3 (S)</td><td rowspan="2">9.6 fmol/µg DNA/ mg</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
</table>

NOTE: All exons of the NUDT15 gene were sequenced. Polymorphisms identified were 415C>T (*2 and *3), 36_37 insGGAGTC (*2 and *6), 416G>A (*4) and 52G>A (*5). *6 was not detected in these Guatemalan, Singaporean and Japanese populations.
</td>
<td></td>
</tr>
<tr>
<td>ref. 21
Yang JJ et al.
Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia.
J Clin Oncol 2015;33:1235-42.
PubMed PMID: 25624441.</td>
<td>4</td>
<td>Genome-wide association study of 657 (discovery cohort) and 371 children (replication cohort) with acute lymphoblastic leukaemia receiving therapy with 6-mercaptopurine (planned dose 75 mg/m^2 per day; dose adjustment based on toxicities (e.g. myelosuppression (leukopenia) and/or infections). Different treatment protocols were used in both cohorts. The discovery cohort received maintenance therapy during 6 months. The replication cohort received continuation therapy. The discovery cohort consisted of children with different genetic ancestries: 205 European, 222 Hispanic, 93 African, 61 Asian and 76 other. The replication cohort consisted of children from the USA.
In the discovery cohort, 6-mercaptopurine prescribed dose was captured monthly. The longitudinal 6-mercaptopurine dose data were summarized into a single overall value for each patient, using a linear mixed-effects model with genetic ancestry and the time point of dose measurement as variables. For each of the NUDT15 phenotypes, the median 6-mercaptopurine dose was similar for all 6 time points.
In the replication cohort, 6-mercaptopurine prescribed dose was assessed longitudinally by phase of therapy during continuation therapy, and summarized using a linear mixed-effects model with genetic ancestry and time point of dose measurement as variables. For patients who stopped therapy early, doses were calculated only up to that time.
The genome-wide significance threshold used was $p < 5x\ 10^{-8}$. Variants found in the discovery threshold were validated in the replication cohort.

Genotyping (*2 and *3):
Discovery cohort:
- 624x NM
- 31x IM (16x Hispanic, 10x East-Asian, 4x other, 1x European)
- 2x PM (1x Hispanic, 1x East-Asian)

Replication cohort:
- 362x NM
- 9x IM

Results:
Genome-wide association study:</td>
<td>Authors' conclusion:
'We describe a germline variant in NUDT15 strongly associated with MP intolerance in childhood ALL, which may have implications for treatment individualization in this disease.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 21, continuation</td>
<td>PM: D
IM: D</td>
<td>
- The polymorphism 415C>T (*2 and *3) was associated with 6-mercaptopurine dose (S).

Median maximum tolerated 6-mercaptopurine dose compared to NM (value for NM as % of planned dose):
<table>
<tr><th>cohort</th><th>ancestry</th><th>PM</th><th>IM</th><th>value for NM</th></tr>
<tr><td rowspan="5">discovery</td><td rowspan="2">all</td><td>x 0.099</td><td>x 0.75</td><td rowspan="2">83.5%</td></tr>
<tr><td colspan="2">S for PM versus IM versus NM</td></tr>
<tr><td>Hispanic</td><td colspan="2">decrease for PM versus IM versus NM (S)</td><td></td></tr>
<tr><td>East-Asian</td><td colspan="2">decrease for PM versus IM versus NM (S)</td><td></td></tr>
<tr><td>European</td><td>-</td><td>NS</td><td></td></tr>
<tr><td>replication</td><td>all</td><td>-</td><td>x 0.78 (S)</td><td>80.1%</td></tr>
</table>

NOTE: For the 39 TPMT IM and for the 1 TPMT PM in the discovery cohort, the median 6-mercaptopurine dose was 0.79x and 0.072x the value for TPMT NM respectively (S), indicating the effects of the NUDT15 variant and TPMT variant to be similar.

NOTE: For 4 patients in the discovery cohort and 1 in the replication cohort being both NUDT15 IM and TPMT IM, the median 6-mercaptopurine dose was 0.47x and 0.70x the value for NM respectively (S), suggesting the effects of the NUDT and TPMT variant to be not fully additive.
</td>
<td>Median maximum tolerated 6-mercaptopurine dose compared to NM at a starting dose of 75 mg/m2 per day:
IM: 76%
PM: 10%</td>
</tr>
<tr>
<td>ref. 22
Yang SK et al.
A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia.
Nat Genet 2014;46:1017-20.
PubMed PMID: 25108385.</td>
<td>4</td>
<td>978 Korean patients with Crohn’s disease were treated with azathioprine or 6-mercaptopurine. Patients who did not experience leukopenia were only included if they received a dose corresponding with azathioprine equivalents ≥ 1 mg/kg per day for at least 8 weeks. 137 patients were excluded due to adverse events. The usual dosing scheme was an increase of a starting dose of 25–50 mg azathioprine equivalents at a rate of 25 mg every 2 to 4 weeks or slower to the target dose of 2.0–3.0 mg/kg/day for azathioprine and 1.0–1.5 mg/kg/day for 6-mercaptopurine as long as there were no leukopenia or other adverse events to prevent further increase of the medication. Decisions regarding dose adjustment or discontinuation of azathioprine or 6-mercaptopurine due to adverse events were made by the physician responsible on a case-by-case basis. The 6-mercaptopurine dose was adjusted to azathioprine equivalents by multiplying by 2.082. The median dose was 1.70 mg/kg azathioprine equivalents per day. Patients were divided over a discovery cohort (n = 340; 33 patients with early leukopenia) and a replication cohort (n = 638; 33 patients with early leukopenia and 280 with late leukopenia). A genome-wide association study was performed in the discovery cohort (genome-wide significance threshold $p < 5x\ 10^{-8}$). All association analyses were performed via logistic regression.
Complete blood count measurements were usually performed biweekly for the first 8 weeks, monthly for the following 1 to 2 months, and then every 2 to 3 months. The interval was shortened if the dose was escalated or if there was a trend towards leukopenia.</td>
<td>Authors’ conclusion:
‘NUDT15 is a pharmacogenetic determinant for thiopurine-induced leukopenia in diverse populations.’</td>
</tr>
</table>

ref. 22, continuation

In addition, 1188 patients with inflammatory bowel disease without TPMT variants from the USA were treated with azathioprine or 6-mercaptopurine (azathioprine equivalents ≥ 1 mg/kg per day for at least 8 weeks in patients without leukopenia; median dose 2.41 mg/kg azathioprine equivalents per day). 73 of these patients developed leukopenia. Logistic regression was performed with adjustment for the top four principal components to control for potential confounding due to population stratification.
Leukopenia was defined as a white blood cell count < 3.0x 10^9/L. Early leukopenia was defined as leukopenia occurring in the first 8 weeks and late leukopenia as leukopenia occurring after the first 8 weeks.
Relevant comedication was not excluded, but logistic regression corrects for confounders.
The two stage design study in Korean patients had a power of 78% to detect a genetic risk variant with a population frequency of 5% and risk effect (OR) of 15 and a power of 90% to detect a risk variant with a population frequency of 5% and an OR of 20 or a population frequency of 10% and an OR of 15.

Genotyping (*2 and *3 in the discovery and replication cohort combined):
- 788x NM
- 176x IM
- 14x PM

Results:

Genome-wide association study:
- The polymorphisms 415C>T (*2 and *3) was associated with early leukopenia (within the first 8 weeks) (S). - *5 was identified by sequencing, but had a low frequency and was not associated with early leukopenia in the discovery and replication cohorts combined (NS).

Results compared to NM:				
cohort		PM (*2/*2, *2/*3, *3/*3)	IM (*1/*2, *1/*3)	value for NM
discovery	early leukopenia	OR = 39.7 (95% CI: 20.0-78.5) (S) for (*2 or *3) versus *1		
replication	early leukopenia	OR = 32.6 (95% CI: 17.4-60.9) (S) for (*2 or *3) versus *1		
discovery + replication	leukopenia	x 4.0 (S)	x 3.0 (S); OR = 9.2 (95% CI: 6.3-13.4)	25.3% of patients
	early leukopenia	x 113 (S)	x 29 (S); OR = 88.1 (95% CI: 37.5-206.9)	0.9% of patients
		89.4% of patients with early leukopenia and 6.8% of patients without leukopenia was carrier of *2 and/or *3		
		With the observed prevalence of 7%, the number		

<table>
<tr><td>ref. 22, continuation</td><td>PM: E
IM: E</td><td>
<table>
<tr><td></td><td></td><td colspan="2">needed to genotype to avoid 1 case of early leukopenia was calculated to be 16.</td><td></td></tr>
<tr><td></td><td>late leukopenia</td><td>-</td><td>x 2.1 (S); OR = 6.3 (95% CI: 4.2-9.4)</td><td>24.4% of patients</td></tr>
<tr><td></td><td>leukopenia grade 3-4</td><td>x 50</td><td>x 3.8</td><td>2.0% of leukopenia cases</td></tr>
<tr><td></td><td></td><td colspan="2">S for PM versus IM versus NM</td><td></td></tr>
<tr><td></td><td>leukopenia grade 4</td><td>85.7% of leukopenia cases</td><td>2.3% of leukopenia cases</td><td>0.0% of leukopenia cases</td></tr>
<tr><td></td><td></td><td colspan="2">S for PM versus IM versus NM</td><td></td></tr>
<tr><td></td><td>time until onset of leukopenia</td><td>x 0.04</td><td>x 0.29</td><td>median 465 days</td></tr>
<tr><td></td><td></td><td colspan="2">S for PM versus IM versus NM</td><td></td></tr>
<tr><td></td><td></td><td colspan="2">Ranges were 9-28 days for PM, 12-3300 days for IM and 21-3705 days for NM.</td><td></td></tr>
<tr><td></td><td>daily dose at onset of leukopenia</td><td>x 0.56</td><td>x 0.69</td><td>median 1.53 mg/kg azathioprine equivalents</td></tr>
<tr><td></td><td></td><td colspan="2">S for PM versus IM versus NM</td><td></td></tr>
<tr><td></td><td></td><td colspan="2">Ranges were 0.50-1.09 mg/kg for PM, 0.26-2.84 mg/kg for IM and 0.14-3.12 mg/kg for NM.</td><td></td></tr>
<tr><td>USA</td><td>leukopenia</td><td colspan="2">OR = 9.5 (S) for (*2 or *3) versus *1</td><td></td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 23
SmPC Puri-Nethol (6-mercaptopurine) 10-05-23.</td><td>0

IM: E
PM: E</td><td colspan="2"><u>Dosing</u>:
Patients with an inherited mutated NUDT15 gene have an increased risk for severe toxicity of 6-mercaptopurine. In general, these patients require a dose reduction, especially if they are homozygous for the NUDT15 variant. Genotypic testing for NUDT15 variants before starting 6-mercaptopurine therapy can be considered. At least, careful monitoring of blood cell counts is necessary.
<u>Warning</u>:
Patients with an inherited mutated NUDT15 gene have an increased risk for severe toxicity of 6-mercaptopurine, such as early leukopenia and alopecia, at conventional doses of thiopurine therapy. In general, these patients require a dose reduction, especially if they are homozygous for the NUDT15 variant. The frequency of NUDT15 c.415C>T has an ethnic variability of approximately 10% in East-Asians, 4% in Latin-Americans, 0.2% in Europeans and 0% in Africans. At least, careful monitoring of blood cell counts is necessary.</td><td></td></tr>
<tr><td>ref. 24
SmPC Imuran (azathioprine) 02-07-21.</td><td>0</td><td colspan="2"><u>Dosing</u>:
Patients with an inherited mutated NUDT15 gene have an increased risk for severe toxicity of 6-mercaptopurine. In general, these patients require a dose reduction, especially if they are homozygous for the NUDT15 variant. Genotypic testing for NUDT15 variants before starting 6-mercaptopurine therapy can be considered. At least, careful monitoring of blood cell counts is necessary.</td><td></td></tr>
</table>

ref. 24, continuation	IM: E PM: E	Warning: Patients with an inherited mutated NUDT15 gene have an increased risk for severe toxicity of 6-mercaptopurine, such as early leukopenia and alopecia, at conventional doses of thiopurine therapy. In general, these patients require a dose reduction, especially if they are homozygous for the NUDT15 variant. The frequency of NUDT15 c.415C>T has an ethnic variability of approximately 10% in East-Asians, 4% in Latin-Americans, 0.2% in Europeans and 0% in Africans. At least, careful monitoring of blood cell counts is necessary. Pharmacodynamics: Recent studies indicate a strong association between the NUDT15 variant NUDT15 c.415C>T [p.Arg139Cys] (also known as NUDT15 R139C [rs116855232]), which is believed to result in loss of function of the NUDT15 enzyme, and thiopurine mediated toxicity, such as leukopenia and alopecia. Patients homozygous for the NUDT15 variant (NUDT15 T risk alleles) have a very high risk of thiopurine toxicity compared to patients homozygous for C. The frequency of NUDT15 c.415C>T has an ethnic variability with an increased risk in the Asian and Latin-American population. Genotypic analysis of the NUDT15 genotype should be performed. The prescribing physician is advised to determine if decreasing the dose is necessary based on the genetic profile of the patient, eventually in combination with the profile of adverse events occurring during treatment. Patients with variants in both the NUDT15 and the TPMT enzyme have significantly less tolerance for thiopurines than patients with risk alleles of only one of these two genes. The exact mechanism of NUDT15 associated thiopurine-related toxicity is not clear.	
ref. 25 SmPC Purinethol (mercaptopurine), USA, 29-12-20.	0	Dose: Evaluate thiopurine S-methyltransferase (TPMT) and nucleotide diphosphatase (NUDT15) status in patients with severe myelosuppression or repeated episodes of myelosuppression. Consider testing for TPMT and NUDT15 deficiency in patients who experience severe bone marrow toxicities or repeated episodes of myelosuppression. *Homozygous deficiency in either TPMT or NUDT15* Patients with homozygous deficiency of either enzyme typically require 10% or less of the recommended dosage. Reduce the recommended starting dosage of Purinethol in patients who are known to have homozygous TPMT or NUDT15 deficiency. *Heterozygous deficiency in TPMT and/or NUDT15* Reduce the Purinethol dose based on tolerability. Most patients with heterozygous TPMT or NUDT15 deficiency tolerate the recommended dosage, but some require a dose reduction based on adverse reactions. Patients who are heterozygous for both TPMT and NUDT15 may require more substantial dose reductions. Warning: Consider testing for TPMT or NUDT15 deficiency in patients with severe myelosuppression or repeated episodes of myelosuppression. TPMT genotyping or phenotyping (red blood cell TPMT activity) and NUDT15 genotyping can iden-	

ref. 25, continuation	IM: E PM: E	tify patients who have reduced activity of these enzymes. Patients with heterozygous or homozygous TPMT or NUDT15 deficiency may require a dose reduction. Clinical pharmacology: *Pharmacogenomics* 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 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%. NUDT15 deficiency is detected in <1% of patients of European or African ancestry. Among patients of East Asian ancestry (i.e., Chinese, Japanese, Vietnamese), 2% have two loss-of-function alleles of the NUDT15 gene, and approximately 21% have one loss-of-function allele. The p.R139C variant of NUDT15 (present on the *2 and *3 alleles) is the most commonly observed, but other less common loss-of-function NUDT15 alleles have been observed.	Dose versus the standard dose: IM: 50-90% PM: 5-10%
ref. 26 SmPC Imuran (azathioprine), USA, 20-12-18.	0	Dose: Consider testing for TPMT and NUDT15 deficiency in patients who experience severe bone marrow toxicities. Early drug discontinuation may be considered in patients with abnormal CBC results that do not respond to dose reduction. *Homozygous deficiency in either TPMT or NUDT15* Because of the risk of increased toxicity, consider alternative therapies for patients who are known to have TPMT or NUDT15 deficiency. *Heterozygous deficiency in TPMT and/or NUDT15* Because of the risk of increased toxicity, dosage reduction is recommended in patients known to have heterozygous deficiency of TPMT or NUDT15. Patients who are heterozygous for both TPMT and NUDT15 deficiency may require more substantial dosage reductions. Warning: Patients with thiopurine S-methyl transferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency may be at an increased risk of severe and life-threatening myelotoxicity if receiving conventional doses of Imuran. In patients with severe myelosuppression, consider evaluation for TPMT and NUDT15. Consider alternative therapy in patients with homozygous TPMT or NUDT15 deficiency and reduced dosages in patients with heterozygous deficiency. Consider genotyping or phenotyping patients for TPMT deficiency and genotyping for NUDT15 deficiency in patients with severe myelosuppression. TPMT and NUDT15 testing cannot substitute for complete blood count (CBC) monitoring in patients receiving Imuran. Clinical pharmacology: Genetic polymorphisms influence TPMT and NUDT15 activity. Several published studies indicate that patients with reduced TPMT or NUDT15 activity receiving usual doses of 6-MP or azathioprine, accumulate excessive cellular concen-	

ref. 26, continuation	IM: E PM: E	trations of active 6-TGNs, and are at higher risk for severe myelosuppression. Because of the risk of toxicity, patients with TPMT or NUDT15 deficiency require alternative therapy or dose modification. NUDT15 deficiency is detected in <1% of patients of European or African ancestry. Among patients of East Asian ancestry (i.e., Chinese, Japanese, Vietnamese), 2% have two loss-of-function alleles of the NUDT15 gene, and approximately 21% have one loss-of-function allele. The p.R139C variant of NUDT15 (present on the *2 and *3 alleles) is the most commonly observed, but other less common loss-of-function NUDT15 alleles have been observed. <u>Adverse reactions</u>: Patients with low or absent TPMT or NUDT15 activity are at increased risk for severe, life-threatening myelosuppression from Imuran.	

Risk group	TPMT IM or PM, use of TPMT inhibitors (aminosalicylates: mesalazine, olsalazine or sulphasalazine, furosemide, acetylsalicylic acid) or xanthine oxidase inhibitors (allopurinol, febuxostat), use of inhibitors of de novo purine synthesis (methotrexate). Note: results regarding the effect of the aminosalicylates 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:

- Studies with at least 175 patients were included before August 2018 and studies and meta-analyses with at least 1300 patient and reporting data separately for IM and PM from August 2018. In addition, genotype-guided studies with at least 70 patients and studies reporting the maximum tolerated dose compared to NM for at least 2 PM and/or at least 25 IM were included. Other publications did not add enough to the evidence.
  The article “Chang JY et al. Genotype-based treatment with thiopurine reduces incidence of myelosuppression in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol 2020;18:2010-18.e2. PMID: 31446180” was not included, because the effect of genotyping was only investigated for NUDT15, CFO and TPMT together and the number of heterozygotes of these genes in the genotype-guided group was 15, 8 and 1 respectively, indicating that in 38% of patients, the azathioprine dose was not escalated due to other reasons than a NUDT15 variant. In addition, initial azathioprine doses were not adjusted for variant allele carriers.
- <u>Other guidelines:</u>
  - Relling MV et al. Clinical Pharmacogenetics Implementation Consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. Clin Pharmacol Ther 2019;105:1095-105. PMID: 30447069, and update guideline on the CPIC-site February 2019 (*9 function has been changed from “uncertain function” to “no function”).
    CPIC defines NUDT15 IM and NUDT15 PM as we do (one or two no function alleles, respectively), but considers only *2, *3 and *9 to be no function alleles. CPIC considers the other alleles, including *4 through *8 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 agnostic genome-wide association studies identified variants in NUDT15 that strongly influence thiopurine tolerance in patients with acute lymphoblastic leukaemia (ALL) and those with inflammatory bowel diseases (Yang 2015 and Yang 2014). In addition, CPIC indicates that patients carrying gene variant 415C>T (allele *2 or *3) showed excessive thioguanine incorporation into DNA and severe myelosuppression (Moriyama 2016), and that in children with ALL, patients homozygous for this variant tolerated only 8% of the standard dose of mercaptopurine, whereas tolerated dose intensity was 63% and 83.5% for those heterozygous and wildtype for this variant, respectively (Yang 2015).
CPIC states that there is substantial evidence linking NUDT15 genotype with phenotypic variability. In addition, retrospective studies strongly indicate that patients with loss-of-function NUDT15 alleles are at excessive risk of thiopurine toxicity if the standard dose is administered (Yang 2014; Tanaka Y et al. Susceptibility to 6-MP toxicity conferred by a NUDT15 variant in Japanese children with acute lymphoblastic leukaemia. Br J Haematol 2015;171:109-15; Chiengthong K et al. NUDT15 c.415C>T increases risk of 6-mercaptopurine induced myelosuppression during maintenance therapy in children with acute lymphoblastic leukemia. Haematologica 2016;101:e24-6; Asada A et al. NUDT15 R139C-related thiopurine leukocytopenia is mediated by 6-thioguanine nucleotide-independent mechanism in Japanese patients with inflammatory bowel disease. J Gastroenterol 2016;51:22-9; Lee YJ et al. NUDT15 variant is the most common variant associated with thiopurine-induced early leukopenia and alopecia in Korean pediatric patients with Crohn's disease. Eur J Gastroenterol Hepatol 2016;28:475-8; Wong FC et al. NUDT15 variant and thiopurine-induced leukopenia in Hong Kong. Hong Kong Med J 2016;22:185-7; Kakuta Y et al. NUDT15 R139C causes thiopurine-induced early severe hair loss and leukopenia in Japanese patients with IBD. Pharmacogenomics J 2016;16:280-5; Ailing Z et al. Further evidence that a variant of the gene NUDT15 may be an important predictor of azathioprine-induced toxicity in Chinese subjects: a case report. J Clin Pharm Ther 2016;41:572-4; Zhu 2016; Soler, A.M. et al. TPMT and NUDT-15 genes are both related to mercaptopurine intolerance in acute lymphoblastic leukaemia patients from Uruguay. Br J Haematol 2018;181:252-5; Yin 2017; Shah SA et al. Nucleoside diphosphate-linked moiety X-type motif 15 C415T variant as a predictor for thiopurine-induced toxicity in Indian patients. J Gastroenterol Hepatol 2017;32:620-4; Tanaka Y et al. Interaction between NUDT15 and ABCC4 variants enhances intolerability of 6-mercaptopurine in Japanese patients with childhood acute lymphoblastic leukemia. Pharmacogenomics J 2018;18:275-80; Zhang 2018).
CPIC indicates that tolerated mercaptopurine dose is correlated with the number of nonfunctional alleles of the NUDT15 gene (Yang 2015 and Yang 2014). CPIC states that, in fact, the degree of thiopurine intolerance (e.g., for mercaptopurine) is largely comparable between carriers of TPMT vs. NUDT15 decreased function alleles (Yang 2015), although there remains a paucity of multi-ethnic studies examining both TPMT and NUDT15 variants. Therefore, CPICs NUDT15 recommendations parallel those for TPMT.
CPIC indicates that starting doses do not need to be altered for NUDT15 normal metabolisers (NUDT15 *1/*1), that reduced starting doses should be considered to minimise toxicity, particularly if the starting doses are high (e.g., 75 mg/m^2 per day for mercaptopurine) for NUDT15 intermediate metabolisers (e.g., NUDT15*1/*3), and that substantially reduced doses (e.g., 10 mg/m^2 per day of mercaptopurine) or the use of an alternative agent should be considered for NUDT15 poor metabolizers (e.g., NUDT15*3/*3) (Moriyama 2016).
In addition, CPIC indicates that, as for TPMT, there is substantial variability in the tolerated thiopurine doses within NUDT15 intermediate metabolisers, with a minority of individuals who do not seem to require significant dose reduction (Yang 2015, Moriyama 2016). Therefore, the genotype-guided prescribing recommendations apply primarily to starting doses; subsequent dose adjustments should be made based on close monitoring of clinical myelosuppression (or disease-specific guidelines). CPIC indicates that, in contrast, a full dose of mercaptopurine poses a severe risk of prolonged hematopoietic toxicity in NUDT15 poor metabolisers and pre-emptive dose reductions are strongly recommended (Zhu 2018 and Ailing Z et al. Further evidence that a variant of the gene NUDT15 may be an important predictor of azathioprine-induced toxicity in Chinese subjects: a case report. J Clin Pharm Ther 2016;41:572-4).
CPIC indicates that the NUDT15 poor metaboliser phenotype is observed at a frequency of about 1 in every 50 patients of East Asian descent, which is more common than the TPMT poor metaboliser phenotype in Europeans, and, thus, genotyping NUDT15 in the Asian populations may be of particular clinical importance. In addition, CPIC indicates that NUDT15 deficiency is also more prevalent in individuals of Hispanic ethnicity, particularly those with high levels of Native American genetic ancestry (Yang 2015).
CPIC classifies all recommendations as strong (i.e. “the evidence is high quality and the desirable effects clearly outweigh the undesirable effects”).
The therapeutic recommendations for 6-mercaptopurine and azathioprine are indicated below:

Dosing recommendations for 6-mercaptopurine and azathioprine by NUDT15 phenotype			
Phenotype	Therapeutic recommendation		Classification of re-
	6-mercaptopurine	azathioprine	

			commen-dation
IM (one no function allele: *1/*2, *1/*3, or *1/*9) or possible IM (one allele with uncertain function (allele other than *1, *2, *3, or *9) and one no function allele: *2, *3, or *9)	Start with reduced starting doses (30-80% of normal dose) if normal starting dose[a] is ≥ 75 mg/m^2/day or ≥ 1.5 mg/kg/day (e.g., start at 22.5–60 mg/m^2/day or 0.45–1.2 mg/kg/day) and adjust doses of mercaptopurine 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 mercaptopurine over other agents[b]. If normal starting dose is already < 75 mg/m^2/day or < 1.5 mg/kg/day, dose reduction may not be recommended.	Start with reduced starting doses (30-80% of normal dose) if normal starting dose[a] is 2-3 mg/kg/day (e.g., 0.6-2.4 mg/kg/day), and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 2-4 weeks to reach steady-state after each dose adjustment[c].	Strong[d]
PM (two no function alleles: *2/*2, *2/*3, *3/*3, *2/*9, *3/*9, or *9/*9)	For malignancy, initiate dose at 10 mg/m^2/day and adjust dose based on 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 mercaptopurine over other agents. For non-malignant conditions, consider alternative non-thiopurine immunosuppressant therapy[e].	For non-malignant conditions, consider alternative non-thiopurine immunosuppressant therapy. For malignant conditions, start with drastically reduced normal daily doses[a] (reduce daily dose by 10-fold) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4-6 weeks to reach steady-state after each dose adjustment[f].	Strong[d]

[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] 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; 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; 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; Relling MV et al. Thiopurine methyltransferase in acute lymphoblastic leukemia. Blood 2006;107:843-4; Sandborn WJ. Rational dosing of azathioprine and 6-mercaptopurine. Gut 2001;48:591-2; Lichtenstein GR et al. Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. Gastroenterology 2006;130:940-87; Krynetski EY et al. Pharmacogenetics of cancer therapy: getting personal. Am J Hum Genet 1998;63:11-6.

[c] 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; Sandborn WJ. Rational dosing of azathioprine and 6-mercaptopurine. Gut 2001;48:591-2; Anstey AV et al. Guidelines for prescribing azathioprine in dermatology. Br J Dermatol 2004;151:1123-32; Lichtenstein GR et al. Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. Gastroenterology 2006;130:940-87.

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

[e] 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; 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; Sandborn WJ. Rational dosing of azathioprine and 6-mercaptopurine. Gut 2001;48:591-2; Lichtenstein GR et al. Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. Gastroenterology 2006;130:940-87.

[f] 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; Sandborn WJ. Rational dosing of azathioprine and 6-mercaptopurine. Gut 2001;48:591-2; Anstey AV et al. Guidelines for prescribing azathioprine in dermatology. Br J Dermatol 2004;151:1123-32; Lichtenstein GR et al. Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. Gastroenterology 2006;130:940-87; Kaskas BA et al. Safe treatment of thiopurine S-methyltransferase deficient Crohn's disease patients with azathioprine. Gut 2003;52:140-2.

Recommendations for patients having also a TPMT 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 TPMT variant are indicated below:

Dosing recommendations for 6-mercaptopurine and azathioprine for patients with a genetically reduced activity for both TPMT and NUDT15		
NUDT15 phenotype	TPMT phenotype	Therapeutic recommendation
IM	IM	Consider dose reduction[a]. See TPMT IM and NUDT15 IM recommendation[b].
IM	PM	Dose reduction recommended[a]. See TPMT PM recommendation.
PM	IM	Dose reduction recommended[a]. See NUDT15 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).

On 7-7-2023, there was not a more recent version of the recommendations present on the CPIC-site.

- Cost-effectiveness:
  - Wei X et al. NUDT15 genetic testing-guided 6-mercaptopurine dosing in children with ALL likely to be cost-saving in China. J. Int J Hematol 2022;115:278-86. PMID: 34709580.

  NUDT15 415C>T testing prior to the initial administration of 6-mercaptopurine in paediatric patients with standard or intermediate risk ALL in China was calculated to both better and cheaper than standard dosing without genetic testing. Genetic testing-guided initial 6-mercaptopurine dosing reduced overall costs by $518.61, prevented 0.221 cases of leukopenia grade III–IV and increased quality-adjusted life-years (QALY) by 0.00136 per patient. Non-genotype-guided maintenance treatment consisted of maintenance treatment with 6-mercaptopurine and methotrexate at a standard dose of 40 mg/m^2 daily and 25 mg/m^2 weekly, respectively. Genotype-guided maintenance treatment consisted of 75% of the standard initial 6-mercaptopurine dose for IM and 25% of the standard initial 6-mercaptopurine dose for PM. Results were robust in one-way analyses and probabilistic sensitivity analyses.

  Cost-effectiveness was calculated over a period of 2 years and from the Chinese medical system perspective. It was assumed that all children with ALL received maintenance treatment with 6-mercaptopurine and methotrexate at a standard dose of 40 mg/m^2 daily and 25 mg/m^2 weekly, respectively, under the assumption of a mean body surface area of 0.80 m^2. And it was assumed that the blood routine of children was examined every 2 weeks for the first 24 weeks of maintenance treatment, then every 4 weeks after 24 weeks given that the 6-mercaptopurine dose had been gradually adjusted to the tolerated dose. Meanwhile, it was assumed that 6-mercaptopurine-induced leukopenia occurs mainly during the early stages of maintenance treatment. All patients who were admitted to the hospital for 2 weeks of leukocyte treatment suffer severe leukopenia early after the use of 6-mercaptopurine. During this period,

chemotherapy was suspended, the 6-mercaptopurine dose was reduced after discharge, and chemotherapy was continued. In reference to the results reported by Tanaka 2018, approximately 1.5% of children in the genetic testing group were assumed to suffer from leukopenia after the initial dose was adjusted in accordance with genotype, and the dose of 6-mercaptopurine is continuously reduced by 1/4 (Tanaka Y et al. Interaction between NUDT15 and ABCC4 variants enhances intolerability of 6-mercaptopurine in Japanese patients with childhood acute lymphoblastic leukemia. Pharmacogenomics J 2018;18:275-80). The event-free survival of children with different genotypes showed no considerable difference after the dose was adjusted to the tolerated amount (Tanaka Y. Susceptibility to 6-mercaptopurine toxicity related with NUDT15 and ABCC4 variants in Japanese childhood acute lymphoblastic leukemia. Rinsho Ketsueki.2017;58:950-6), so it was assumed that comparable therapeutic effects can be achieved in children by adjusting the starting dose in accordance with genotype. Tanaka 2017 also showed that no remarkable differences existed in the proportion of 6-mercaptopurine treatment discontinuations for patients with different genotypes, so children who discontinued treatment were excluded from the analysis in this cost-effectiveness study. Direct and indirect medical costs were included in the calculation. Direct medical costs included the cost of treatment drugs, examination, genetic testing, and the other direct medical costs of 6-mercaptopurine-induced leukopenia grade 3-4. Indirect medical costs mainly included the loss of parental labour, transportation costs, and time costs resulting from the treatment of leukopenia grade 3-4. The estimated costs of the not-genotype-guided and NUDT15-guided treatments for 2 years were € 3,676.71/patient and € 3,158.10./patient, respectively. The total average QALYs in the not-genotype-guided and NUDT15-guided treatments for 2 years were 1.51815 and 1.51951, respectively. The avoided severe leukopenia cases in the not-genotype-guided and NUDT15-guided treatments were 0.700 and 0.921, respectively. Cost of standard dose 6-mercaptopurine was $0.25/day, cost of methotrexate was $4.06/week, cost of laboratory examination was €81.94, indirect medical cost was $446.93, cost of severe leukopenia was $2,369.70, NUDT15 genotyping cost was $71.06.
The prevalence of PM (10.3%) and IM (36.4%) in Chinese was derived from Liu 2018. This corresponds to a variant allele frequency of 32%.
One-way sensitivity analysis (variation of one input parameter at a time) showed the cost of severe leukopenia to have the strongest effect on total costs, followed by discount rate and indirect medical and genotyping costs.
Probabilistic sensitivity analyses (1000 simulations, variation of all parameter) showed the cost-effectiveness probability of genotype-guided therapy to be 100% using a willingness-to-pay threshold of $26,508/QALY (i.e. 3 times the per capita gross domestic product of $8,836 in 2017). In 67.1% of these simulations, genotype-guided therapy was also better than non-genotype-guided therapy. Note: the lowest variant allele frequency used in the probabilistic sensitivity analyses was 22%, which is still 11-fold higher than the *3 allele frequency of 0.2% reported in Europeans.

- Zeng D et al. Cost-effectiveness analysis of genotype screening and therapeutic drug monitoring in patients with inflammatory bowel disease treated with azathioprine therapy: a Chinese healthcare perspective using real-world data. Ann Transl Med 2021;9:1138. PMID: 34430579.
For azathioprine-treated Chinese patients with inflammatory bowel disease, prevention of severe thiopurine–induced leukopenia by NUDT15-guided treatment was calculated to be both better and cheaper compared to not-genotype guided treatment. NUDT15-guided treatment saved €112.88 per patient and provided an additional 0.00430 quality-adjusted life years (QALYs) per patient. NUDT15-guided treatment consisted of normally dosed azathioprine treatment for NM patients, 50% of the normal azathioprine dose for IM patients, and an alternative drug (TNF-α inhibitors or methotrexate) for PM patients.
In this Chinese patient group, NUDT15- and TPMT-guided treatment was cost-effective compared to NUDT15-guided treatment. Compared to NUDT15-guided treatment, additional cost for NUDT15- and TPMT-guided treatment were $3,929.54 per QALY gained, which is below the willingness-to-pay level of $30,425/QALY in China.
Therapeutic drug monitoring reduced the cost and increased the QALY even further.
Cost-effectiveness was calculated over a period of 1 year (because most severe cases of azathioprine-induced myelotoxicity occur within 1 year after initiation of treatment) and from the Chinese health care perspective. Only direct medical costs (costs of the genetic test and costs of treatment) were included in the calculation. The mean estimated costs of the not-genotype-guided and NUDT15-guided treatments for 1 year were €721.82/patient and €608.94/patient, respectively. The total of QALYs gained in the not-genotype-guided and NUDT15-guided treatments for 1 year were 0.87873 and 0.88303, respectively. Cost of standard dose azathioprine was $243/year (including prescription and laboratory test fees (i.e., complete blood count and blood chemistry for liver enzymes)), cost of 50%-reduced-dose azathioprine was $182/year (including prescription and laboratory test fees (i.e., complete blood count and blood chemistry for liver enzymes)), cost of methotrexate was $211/year (including prescription and laboratory test fees (i.e., complete blood count and blood chemistry for liver enzymes)), cost of TNF-α inhibitor use (including office visits, and hospitalisations for infusion) was $20,457/year, cost of treatment of azathioprine-induced severe myelotoxicity was $363, NUDT15 genotyping cost was $51.5. Prevalence of NUDT-

15 genotypes, efficacy of genotype screening, and the probability of severe myelotoxicity (absolute neutrophil count <0.5x10^9/L or pancytopenia needing hospital admission for treatment) were retrospectively derived from 391 patients with inflammatory bowel disease. The frequency of NUDT15 PM was 1.6% in this Chinese cohort (corresponding to an inactive allele frequency of approximately 12.6%).The frequency of TPMT PM was 0.037% (corresponding to an inactive allele frequency of approximately 1.9%).

- Zarca K et al. Cost-effectiveness analysis of pretreatment screening for NUDT15 defective alleles. Pharmacogenet Genomics 2020;30:175-83. PMID: 32433339.
  The additional costs per case of severe myelosuppression averted, were calculated for French adult patients with inflammatory bowel disease for whom azathioprine was considered suitable as first-line monotherapy. With a low cost-effectiveness threshold, combined screening for NUDT15 and TPMT defective alleles was calculated to be cost-effective compared to TMPT screening alone in patients of Asian descent, but to be unrealistic from a cost-effectiveness point of view in Whites. Combined TPMT/NUDT15 genotyping compared with TPMT genotyping had additional cost of € 7,491,281 per severe myelotoxicity averted in Whites, compared to € 619 in Asians.
  The additional cost of a next-generation sequencing based screening strategy is disproportionally high compared with genotyping, irrespective of ethnic descent (additional cost of € 11,538,651 and € 48,438 per severe myelotoxicity averted in Whites and Asians, respectively).
  The probability of additional genotyping of NUDT15 to be cost-effective in Asian patients was 99% if the decision-maker is willing to pay € 7500. The additional cost of double genotyping in Asians were highly dependent on the prevalence of severe myelotoxicity, the sensitivity of combined genotyping, and the cost of myelotoxicity. Should any of these three values be increased, the additional cost would drop.
  Targeted genotyping was for NUDT15 *2, *3, and *9 and TPMT*2, *3A, *3B and *3C. PM were given alternative therapy (anti-TNF-α or a reduced (10%) dose of azathioprine). TPMT IM received normal azathioprine therapy.
  Cost-effectiveness was calculated over a period of 1 year (because the vast majority of azathioprine-related severe myelotoxicities occur within 1 year of the initiation of treatment) and from the French health care provider perspective. Severe myelosuppression was defined as an absolute neutrophil count below 0.5x10^9/L, a level associated with a significant risk of infection which should be managed on an inpatient basis, or as pancytopenia requiring hospitalisation. Only direct medical costs (costs of the diagnostic test and costs of treatment) were included in the calculation. The mean estimated cost of the TPMT genotyping, TPMT/NUDT15 genotyping and TPMT/NUDT15 next generation strategies for 1 year were € 488.4126/patient, € 598.3067/patient and € 1261.0278/patient, respectively, in Whites and € 981.64/patient, € 990.12/patient and € 1653.03/patient, respectively, in Asians. The mean averted severe myelosuppression cases per 10,000 patients by TPMT genotyping, TPMT/NUDT15 genotyping and TPMT/NUDT15 next generation strategies for 1 year were € 43.3, 43.4 and 43.7, respectively, in Whites and 3.6, 140.5 and 140.5, respectively, in Asians. Cost for azathioprine (including monthly cell blood counts and liver tests) was € 300/year, cost for TNF-α inhibitors (including office visits, and hospitalisations for infusion) was € 4200/year, cost of severe myelotoxicity (requiring hospitalization) was € 7000, NUDT15 genotyping cost and TPMT genotyping cost were € 110, combined NUDT15/TPMT genotyping cost was € 220, and next-generation sequencing cost <20kb was € 880. Prevalence rates of NUDT15 and TPMT genotypes/phenotypes, sensitivity and specificity of NUDT15 and TPMT testing, and incidence of severe myelosuppression from previous reports were used: a prevalence of TPMT PM of 0.433% in Whites and 0.037% in Asians, a prevalence of NUDT15 PM of 0.002% in Whites and 1.368% in Asians, and a probability of severe myelosuppression of 1% in Whites and 5.3% in Asians. The authors assumed that 100% of NUDT15 or TPMT PM would develop severe myelosuppression if treated with full-dose azathioprine. They hypothesized that azathioprine treatment was discontinued and replaced by anti-TNF-α or reduced doses of azathioprine if patients developed severe bone marrow toxicity. Furthermore, they assumed that the only side effect of azathioprine was myelotoxicity.

National Cancer Institute Common Toxicity Criteria (NCI-CTC)

	grade 1 = B	**grade 2 = C**	**grade 3 = D**	**grade 4 = E**	**grade 5 = F**
Diarrhoea	Increased stool frequency by < 4; slight increase in stoma output	Increased stool frequency by 4-6; moderate increase in stoma output; no effect on daily activities	Increased stool frequency by ≥ 7; incontinence; IV fluid ≥ 24 hours; hospitalisation; severe increase in stoma output; effect on daily activities	Life-threatening consequences (e.g. haemodynamic collapse)	Death
Neutropenia	> 1.5x10^9/L	< 1.5-1.0x10^9/L	< 1.0-0.5x10^9/L	< 0.5x10^9/L	Death

Leukopenia	> $3.0x10^9$/L	< 3.0-$2.0x10^9$/L	< 2.0-$1.0x10^9$/L	< $1.0x10^9$/L	Death
Thrombocytopenia	> $75x10^9$/L	75-$50x10^9$/L	50-$25x10^9$/L	< $25x10^9$/L	Death
Febrile neutropenia	-	-	Present	Life-threatening consequences (e.g. septic shock, hypotension, acidosis, necrosis)	Death

ULN = upper limit of normal

Date of literature search: 6 July 2023.

	Phenotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	IM	4 E	yes	yes	25 September 2023
	PM	4 E	yes	yes	

Mechanism:
NUDT15 reverses the final step in the formation of the active metabolite of 6-mercaptopurine and its precursor azathioprine. It converts 6-thiodeoxyguanosine triphosphate (6-thio-dGTP), which is incorporated into DNA, into 6-thiodeoxyguanosine monophosphate (6-thio-dGMP). For this reason, lower metabolic activity of NUDT15 leads to increased intracellular concentrations of the active metabolite 6-thio-dGTP. This increases the risk of adverse events such as myelosuppression.

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**	
		European, African	**Asian, (Latin-) American**
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+	6+	7+
Corresponding Clinical Implication Score:		Essential	Essential