NUDT15: azathioprine/6-mercaptopurine 7035/7036

ALL = acute lymphoblastic leukaemia, CI = confidence interval, DNA-TG = DNA-incorporated 6-thioguanine-nucleotides, EM = extensive metaboliser (*1/*1) (normal NUDT15 enzyme activity), HR = hazard ratio, IM = intermediate metaboliser (*1/*2, *1/*3, *1/*4, *1/*5 *1/*6) (reduced 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 confirm that patients with genetically reduced NUDT15 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. 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. Five included studies (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 five 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. Four of the studies used for dose calculations concern acute lymphoblastic leukaemia (ALL) therapy (Yi 2017, Kim 2017, Chao 2017, Liang 2016 and Yang 2015). The fifth study concerns inflammatory bowel disease therapy, but provides only data for IM (Chao 2017).

PM: The weighted mean of the calculated dose adjustment for 13 PM from 4 ALL studies is a dose reduction to 20% (10-24%, median 20%). In ALL therapy, 6-mercaptopurine and methotrexate dose are usually lowered alternately in case of leukopenia or other side effects. This indicates that using 20% 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. 10% of the normal dose. 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 283 IM from 5 studies is a dose reduction to 71% (65-78%, median 70%). In ALL therapy, 6-mercaptopurine and methotrexate dose are usually lowered alternately in case of leukopenia or other side effects. In addition, in the only inflammatory bowel disease study,

the tolerated dose was only calculated in patients tolerating azathioprine or 6-mercaptopurine for more than 6 months (60% of the EM, 47% of the IM and none of the PM). 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. Two studies comparing NUDT15 and TPMT directly observed a similar effect of variants of these two genes on the tolerated thiopurine dose (Liang 2016 and Yang 2015). Because TPMT variants do not affect the ratio of the different 6-TGNs, the dose adjustment for TPMT IM could also be calculated based on the change in total 6-TGN level (see the corresponding risk analysis). Thus, the calculated dose adjustment was also based on the effect on thiopurine metabolism in all patients. For these reasons, the KNMP pharmacogenetics working group decided to recommend the same initial dose for NUDT15 IM as for TPMT IM, i.e. 50% of the normal dose. 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 EM and IM patients (final median dose approximately 80% and 60% of the starting dose for EM 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 on 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 Dutch 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 9 studies and 2 meta-analyses. 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 EM (ref. 1, 2 and 5). 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 EM 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 $10 < NNG \leq 100$). 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 $100 < NNG \leq 1000$).

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).

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

<table>
<tr><th>Source</th><th>Code</th><th>Effect</th><th>Comments</th></tr>
<tr>
<td>ref. 1
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 Mar 8 [Epub ahead of print].
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 EM - 37x IM+PM (31x IM, 6 PM)
*2 and *6: - 172x EM - 16x IM+PM
*5: - 185x EM - 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 heterozygous 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</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>

<table>
<tr><td>ref. 1, continuation</td><td></td><td>
<table>
<tr><td></td><td></td><td>dose (i.e. 2.5 mg/m^2 every two days).</td><td></td></tr>
<tr><td></td><td>*2 and *6</td><td>x 3.2 (S)
NS after correction for the 415C>T polymorphism (identifying *2 and *3) in multivariate analysis.</td><td>22%</td></tr>
<tr><td></td><td>*5</td><td>NS</td><td>25%</td></tr>
<tr><td></td><td>Phe52Leu</td><td>NS
The only carrier developed leukopenia and the final 6-mercaptopurine dose was 80% of the normal dose.</td><td>25%</td></tr>
<tr><td></td><td>Glu115Gly + frameshift</td><td>NS
The only carrier developed leukopenia and the final 6-mercaptopurine dose was 67% of the normal dose.</td><td>25%</td></tr>
<tr><td></td><td>Gly161Arg</td><td>NS
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.</td><td>25%</td></tr>
<tr><td>% of patients with hepatotoxicity</td><td>*2 and *3</td><td>NS</td><td>21%</td></tr>
<tr><td></td><td>*2 and *6</td><td>NS</td><td>19%</td></tr>
<tr><td></td><td>*5</td><td>NS</td><td>19%</td></tr>
<tr><td></td><td>Phe52Leu</td><td>NS</td><td>20%</td></tr>
<tr><td></td><td>Glu115Gly + frameshift</td><td>NS</td><td>20%</td></tr>
<tr><td></td><td>Gly161Arg</td><td>NS</td><td>20%</td></tr>
</table>
NOTE: Genotyping was by sequencing of NUDT15.
</td><td></td></tr>
<tr><td>ref. 2
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</td><td>Meta-analysis of 7 studies investigating the effect of *2 and *3 on thiopurine-induced leukopenia (1138 patients in total; 827 EM, 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.
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,</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</td></tr>
</table>

<table>
<tr>
<td>ref. 2, continuation</td>
<td>IM: D
PM: D</td>
<td>Chiengthong 2016, Zhu 2016, Kakuta 2015, 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. Otherwise, a fixed-effects model was used.

Results:
<table>
<tr><td colspan="2">Percentage of patients with leukopenia compared to EM (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>treatment may be useful to limit leukocytopenia.’</td>
</tr>
<tr>
<td>ref. 3
Yi ES et al.
NUDT15 variants cause hematopoietic toxicity with low 6-TGN levels in children with acute lymphoblastic leukemia. Cancer Res Treat 2017 Sep 13 [Epub ahead of print]. PubMed PMID: 28903549.</td>
<td>4

IM: D</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 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 EM, 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 EM
- 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 EM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for EM</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></td><td>x 5.5 (S)</td><td>x 1.04 (NS)</td><td>14.6%</td></tr>
</table>
</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.’

Average dose 3-12 months after therapy start compared to EM at a starting dose of 50 mg/m^2 per day:
IM: 78%
PM: 24%</td>
</tr>
</table>

<table>
<tr>
<td>ref. 3, continuation</td>
<td>PM: E</td>
<td>
<table>
<tr><td>% of patients with febrile neutropenia</td><td colspan="2">S for PM versus IM versus EM</td><td></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 EM</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 EM</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 EM</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 EM</td></tr>
</table>
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. 4
Kim H et al.
APEX1 polymorphism and mercaptopurine-related early onset neutropenia in pediatric acute lymphoblastic leukemia.
Cancer Res Treat 2017 Sep 4 [Epub ahead of print].
PubMed PMID: 28882023.</td>
<td>4</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</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.'</td>
</tr>
</table>

<table>
<tr>
<td>ref. 4, continuation</td>
<td>IM: D

PM: E</td>
<td>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 EM, 97% of IM and all PM).

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

Results:
<table>
<tr><td colspan="4">Results compared to EM:</td></tr>
<tr><td></td><td colspan="2">PM</td><td>IM</td><td>value for EM</td></tr>
<tr><td rowspan="3">maximum tolerated 6-mercaptopurine dose</td><td colspan="2">x 0.19</td><td>x 0.65</td><td rowspan="3">26.9 mg/m^2 per day</td></tr>
<tr><td colspan="3">S for PM versus IM versus EM</td></tr>
<tr><td colspan="3">Note: The methotrexate dose reduction was also higher for PM versus IM versus EM (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 EM.</td></tr>
<tr><td rowspan="3">% of patients with neutropenia grade 4 in the first 720 days</td><td colspan="2">x 1.5 (S)</td><td>x 1.1 (NS)</td><td rowspan="3">65.0%</td></tr>
<tr><td colspan="3">S for PM versus IM versus EM</td></tr>
<tr><td colspan="3">Multivariate analysis showed PM to be an independent risk factor for neutropenia grade 4 (HR = 11.4 (95% CI: 3.4-39.0) (S), but IM not (NS).</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>Maximum tolerated 6-mercaptopurine dose compared to EM at a starting dose of 50 mg/m^2 per day:
IM: 65%
PM: 19%</td>
</tr>
<tr>
<td>ref. 5
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</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</td>
</tr>
</table>

<table>
<tr>
<td>ref. 5, continuation</td>
<td></td>
<td>more than 6 months (n = 405; 60% of the EM, 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 EM
- 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 EM</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="5">*2 and *3</td><td>x 6.2</td><td>x 2.9</td><td rowspan="5">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 showed 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).</td></tr>
<tr><td colspan="2">The sensitivity of *2 and *3 for predicting leukopenia was 49.2%, with a specificity of 84.1%.</td></tr>
<tr><td rowspan="5">*2 and *6</td><td>x 4.8</td><td>x 2.4</td><td rowspan="5">20.8%</td></tr>
<tr><td colspan="2">S for homozygous versus heterozygous versus (no *2 and *6)</td></tr>
<tr><td colspan="2">OR = 3.52 (95% CI: 2.26-5.49) (S)</td></tr>
<tr><td colspan="2">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%</td></tr>
</table>
</td>
<td>c.415C>T in Chinese patients with IBD. Treatment monitoring by NUDT15 variants may be promising in individualized therapy.’</td>
</tr>
</table>

ref. 5, continuation				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 grade 1	*2 and *3	x 0	x 0.71	58.9%	
				S for (*2 and/or *3) versus (no *2 and *3)			
			*2 and *6	x 2.0	x 0.72	51.1%	
				S for (*2 and/or *6) versus (no *2 and *6)			
			*5	-	NS	48.5%	
		% of leukopenia being early (week 0-8)	*2 and *3	x 3.3	x 1.4	30.0%	
				S for homozygous versus heterozygous versus (no *2 and *3)			
			*2 and *6	x 0	x 1.1	38.5%	
				S for (*2 and/or *6) versus (no *2 and *6)			
			*5	-	x 2.0 (S)	37.9%	
		% of leukopenia being middle (week 8-24)	*2 and *3	x 0	x 1.4	22.2%	
				S for (*2 and/or *3) versus (no *2 and *3)			
			*2 and *6	x 0	x 1.0	24.4%	
				S for (*2 and/or *6) versus (no *2 and *6)			
			*5	-	NS	24.3%	
		% of leukopenia being late (> 24 weeks)	*2 and *3	x 0	x 0.58	47.8%	
				S for (*2 and/or *3) versus (no *2 and *3)			
			*2 and *6	x 2.7	x 0.85	37.0%	Median tolerated dose compared to EM at a starting dose of azathioprine 2 mg/kg per day: IM: 67%
				S for (*2 and/or *6) versus (no *2 and *6)			
			*5	-	NS	37.9%	
		median daily dose after	all	-	x 0.67 (S)	1.6 mg/kg	
			*2 and *3	-	x 0.65 (S)	1.6 mg/kg	

<table>
<tr><td>ref. 5, continuation</td><td></td><td>
<table>
<tr><td rowspan="2">more than 6 months</td><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></td></tr>
<tr><td>ref. 6
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

PM: D
IM: D</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 EM, 38 IM and 8 PM) and 4 inflammatory bowel disease publications with in total 1527 patients (1218 EM, 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 EM, 165 IM and 9 PM) and 4 inflammatory bowel disease publications with in total 1343 patients (1082 EM, 239 IM and 22 PM).
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 Mehh-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.

Results:
<table>
<tr><td colspan="4">Results compared to EM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for EM</td></tr>
<tr><td rowspan="2">% 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 rowspan="2">24.5%</td></tr>
<tr><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></tr>
<tr><td rowspan="3">tolerated dose</td><td colspan="2">x 0.72 (95% CI: 0.66-0.79) (S)</td><td rowspan="3"></td></tr>
<tr><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 EM respectively.</td></tr>
<tr><td colspan="2">The dose reduction in 23 PM was a dose reduction to 43% of the dose for EM and the dose</td></tr>
</table>
</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 NUDT15 polymorphisms for clinical regimen, rs116855232 should be considered as a highly credible pharmacogenetic indicator for thiopurines using espcially is Asians.'

(Tolerated dose compared to EM:
IM: 78%
PM: 43%)</td></tr>
</table>

<table>
<tr><td>ref. 6, continuation</td><td></td><td>
<table>
<tr><td></td><td>reduction in 386 IM a dose reduction to 78% of the dose for EM (significance not determined).</td><td></td></tr>
<tr><td colspan="3">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>
<tr><td colspan="3">For both meta-analyses, there were no indications for publication bias.</td></tr>
<tr><td colspan="3">The calculated ORs were similar after removing each study one at a time from the meta-analyses.</td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 7
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 EM
- 70x IM
- 2x PM

Results:
<table>
<tr><td colspan="4">Results compared to EM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for EM</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 EM</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 EM (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 EM at a starting dose of 60 mg/m^2 per day:
IM: 70%
PM: 21%</td></tr>
</table>

<table>
<tr>
<td>ref. 8
Zhu X et al.
NUDT15 polymorphisms are better than thiopurine S-methyltransferase as predictor of risk for thiopurine-induced leukopenia in Chinese patients with Crohn's disease.
Aliment Pharmacol Ther
2016;44:967-75.
PubMed PMID: 27604507.</td>
<td>4

PM: E
IM: D</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 < $1.5x10^9$/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 EM
- 53x IM
- 4x PM

Results:
<table>
<tr><td colspan="4">Results compared to EM:</td></tr>
<tr><td></td><td>PM</td><td>IM</td><td>value for EM</td></tr>
<tr><td rowspan="3">% of patients with leukopenia</td><td>x 7.8</td><td>x 5.3</td><td rowspan="3">12.8%</td></tr>
<tr><td colspan="2">S for PM versus IM versus EM</td></tr>
<tr><td colspan="2">PM had leukopenia grade 3 or 4.</td></tr>
<tr><td rowspan="2">% of patients with early leukopenia (week 0-8)</td><td>x 24</td><td>x 6.9</td><td rowspan="2">4.1%</td></tr>
<tr><td colspan="2">S for PM versus IM versus EM</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 EM)</td><td>2.6%</td></tr>
<tr><td rowspan="2">correlation of 6-TGN concentrations with</td><td colspan="2">no</td><td rowspan="2">yes</td></tr>
<tr><td colspan="2">The mean 6-TGN</td></tr>
</table>
</td>
<td>Authors’ conclusion:
‘In Chinese patients, it is strongly recommended to detect NUDT15 genotype rather than TPMT before initiating thiopurine 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. 8, continuation</td><td></td><td>
<table>
<tr><td>leukopenia</td><td>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 EM 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. 9
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 EM
- 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 EM:</td></tr>
<tr><td></td><td>country</td><td>PM</td><td>IM</td><td>value for EM</td></tr>
<tr><td rowspan="7">median maximum tolerated 6-mercaptopurine dose</td><td rowspan="2">all</td><td colspan="2">decrease for PM versus IM versus EM (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 EM (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 EM (S)</td></tr>
<tr><td>Japan</td><td></td><td>decrease (S)</td><td></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. 9, continuation</td><td></td><td>
<table>
<tr><td></td><td></td><td colspan="2">decrease for PM versus IM versus EM (S)</td><td></td></tr>
<tr><td rowspan="3">median dose-corrected DNA-TG level</td><td rowspan="3">Singapore + Japan</td><td colspan="2">increase for PM versus IM versus EM (S)</td><td rowspan="3"></td></tr>
<tr><td colspan="2">There was no significant heterogeneity between the two patient groups.</td></tr>
<tr><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></tr>
<tr><td rowspan="2">Singapore</td><td>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 EM</td></tr>
<tr><td rowspan="2">Japan</td><td>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 EM</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. 10
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: — Replication cohort:
- 624x EM — - 362x EM
- 31x IM (16x Hispanic, 10x East- — - 9x IM</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. 10, continuation</td>
<td>PM: D
IM: D</td>
<td>
Asian, 4x other, 1x European)
- 2x PM (1x Hispanic, 1x East-Asian)

Results:
<table>
<tr><td>Genome-wide association study:</td></tr>
<tr><td>- The polymorphism 415C>T (*2 and *3) was associated with 6-mercaptopurine dose (S).</td></tr>
</table>
<table>
<tr><td colspan="5">Median maximum tolerated 6-mercaptopurine dose compared to EM (value for EM as % of planned dose):</td></tr>
<tr><td>cohort</td><td>ances-try</td><td>PM</td><td>IM</td><td>value for EM</td></tr>
<tr><td rowspan="5">disco-very</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 EM</td></tr>
<tr><td>Hispa-nic</td><td colspan="2">decrease for PM versus IM versus EM (S)</td><td></td></tr>
<tr><td>East-Asian</td><td colspan="2">decrease for PM versus IM versus EM (S)</td><td></td></tr>
<tr><td>Euro-pean</td><td>-</td><td>NS</td><td></td></tr>
<tr><td>replica-tion</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 EM 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 EM 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 EM at a starting dose of 75 mg/m2 per day:
IM: 76%
PM: 10%</td>
</tr>
<tr>
<td>ref. 11
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</td>
<td>Authors’ conclusion:
‘NUDT15 is a pharmacogenetic determinant for thiopurine-induced leukopenia in diverse populations.’</td>
</tr>
</table>

ref. 11, continuation

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.
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 EM
- 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 EM:				
cohort		PM (*2/*2, *2/*3, *3/*3)	IM (*1/*2, *1/*3)	value for EM
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		

<table>
<tr><td>ref. 11, continuation</td><td>PM: E
IM: E</td><td>
<table>
<tr><td></td><td></td><td colspan="2">leukopenia and 6.8% of patients without leukopenia was carrier of *2 and/or *3</td><td></td></tr>
<tr><td></td><td></td><td colspan="2">With the observed prevalence of 7%, the number 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 EM</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 EM</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 EM</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 EM.</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 EM</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 EM.</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. 12
SmPC Puri-Nethol (6-mercaptopurine) September 2017 and other[a].</td><td>0

IM: E
PM: E</td><td colspan="2">Dosing:
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.
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</td><td></td></tr>
</table>

ref. 12, continuation		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.	

[a] SmPC Imuran (azathioprine) 29-11-17.

Risk group	TPMT IM or PM

Comments:

- Only studies with at least 175 patients were included. Smaller studies did not add enough to the evidence.

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 June 2018.

	Phenotype	**Code**	**Gene-drug interaction**	**Action**	**Date**
KNMP Pharmacogenetics Working Group decision	IM	4 E	yes	yes	4 March 2019
	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 genotype 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