TPMT: azathioprine/6-mercaptopurine 1905/1906

ALL = acute lymphoblastic leukaemia, AZA = azathioprine, CI = 95% confidence interval, Cl_{or} = oral clearance, cytostat = cytostatic agent, IBD = inflammatory bowel disease, IM = intermediate metaboliser (reduced TPMT enzyme activity; *1/variant), imm sup = immunosuppressant, 6-MMP = 6-methylmercaptopurine, 6-MP = 6-mercaptopurine, MR = metabolic ratio, NM = normal metaboliser (normal TPMT enzyme activity; *1/*1), NS = non-significant, OR = odds ratio, PM = poor metaboliser (absent or severely reduced TPMT enzyme activity; variant/ variant), RBC = (relating to) red blood cells, S = significant, TDM = therapeutic drug monitoring, 6-TG = thioguanine, 6-TGN = 6-thioguanine nucleotide, TPMT = thiopurine S-methyltransferase, UM = ultra-rapid metaboliser (increased TPMT enzyme activity, not genetically determined), XO = xanthine oxidase

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

Brief summary and justification of choices:
Azathioprine is converted in the body to mercaptopurine. TPMT converts mercaptopurine primarily to inactive metabolites. The enzyme therefore reduces the percentage of mercaptopurine that is converted to the active metabolite. Genetic variations in TPMT lead to decreased enzyme activity, which results in an increased percentage of azathioprine and mercaptopurine that is converted to the active metabolite. Therapeutic and toxic concentrations of the active metabolite are therefore reached at lower doses.
All 3 meta-analyses and 10 of the 17 studies included in the risk analysis and investigating an association between adverse events and genetically reduced TPMT enzyme activity in patients using ≥ 1.5 mg/kg azathioprine or ≥ 0.75 mg/kg mercaptopurine per day confirm that patients with genetically reduced TPMT enzyme activity (intermediate metabolisers (IM) and poor metabolisers (PM)) have an increased risk for leukopenia and/or dose reduction due to adverse events like leukopenia (Dong 2010, Higgs 2010, Booth 2011, Relling 1999, Evans 2001, Black 1998, Pandya 2002, Ansari 2002, Fabre 2004, Zelinkova 2006, Sheffield 2009, Lennard Br J Haematol 2015;169:228-40, and Liu 2017). In addition, the largest of the two studies investigating genotype-guided treatment showed genotype-guided dosing to reduce leukopenia in IM (Coenen 2015).
Results of studies investigating an association between effectiveness and genetically reduced TPMT enzyme activity were contradictory, so there is no evidence that the increased risk in leukopenia is compensated for by a better effectiveness of treatment (Fabre 2004, Stanulla 2005, Gardiner 2008, Levinsen 2014, Lennard Br J Haematol 2015;169:228-40, and Lennard Br J Haematol 2015;170:550-8).
This is why the KNMP Pharmacogenetics Working Group decided that this concerns a gene-drug interaction and that action is required, namely to reduce the dose (yes/yes-interactions).
You can find a detailed overview of the observed clinical and kinetic effects per phenotype in the background information text of the gene-drug interactions on the KNMP Kennisbank. You might also have access to this background text via your pharmacy or physician electronic decision support system.
Substantiation of the (dose) recommendation for each phenotype is provided below.
<u>Justification of the (dose) recommendation per phenotype</u>
If possible, doses are corrected for concentrations of the active metabolite (6-TGN). In the case of IM, doses are only included in the calculation if correction for 6-TGN concentration was possible, or if the study found no difference between NM and IM in the frequency of adverse events. All doses have been included for PM, because there was insufficient information available.
PM: Particularly in the case of PM, there is a clear link between the lower TPMT activity and the increased risk of adverse events. Patients with the PM phenotype are virtually always intolerant to the standard dose of azathioprine or 6-mercaptopurine. Out of the 45 PM cases in the literature, only 1 was able to tolerate the standard dose (Andersen 1998, McLeod 1999, Relling 1999, Evans 2001, Ansari 2002, Gearry 2003, Kaskas 2003, Schaeffeler 2003, Gilissen 2004, Kurzawski Ther Drug Monit 2005, Kurzawski Transpl Int 2005, Stanulla 2005, Gardiner 2006, Zelinkova 2006, Newman 2011, Kim 2012, Lee 2013, Belen 2014, Demlova 2014, Kim 2014, Coenen 2015, Lennard Br J Haematol 2015;169:228-40, Lennard Br J Haematol 2015;170:550-8, Liu 2017). The dose adjustment calculated based on data from the literature is a reduction to 2.2%-124% of the standard dose (weighted mean 13.5%, median 10.5%) (n = 37). If the tolerant PM is excluded, then the calculated dose adjustment is a reduction to 2.2%-15.5% of the standard dose (weighted mean 10.4%, median 10.3%) (n = 36). This was translated to 10% to be more achievable in clinical practice.

Adjustment of the initial dose should be guided by toxicity and effectiveness.

IM: Contradictory results were found regarding the influence of the IM phenotype on the effectiveness of the therapy. In addition, when used as an oncolytic drug, there are reports of an increase in secondary malignant tumours in IM (see Comments/Dose recommendations in reviews/articles at the end of this risk analysis and Levinsen 2014). Lennard found an non-significantly increased effectiveness for *1/*3A and a decreased effectiveness for *1/*3C in a study involving 709 children, but found no influence of the IM phenotype or any of the IM genotypes on effectiveness in a study involving 2387 patients, aged 1-25 years, and a more effective treatment protocol (Lennard Br J Haematol 2015;169:228-40, and Lennard Br J Haematol 2015;170:550-8). Levinsen 2014 found no difference in effectiveness in 674 children and no difference in new tumours between IM at 67% of the standard initial dose and NM at the standard initial dose. Gardiner 2008 found no difference in clinical outcome between IM and NM in a group of 52 patients with inflammatory bowel disease. Fabre 2004 found no difference in the percentage of patients with at least one acute rejection episode between IM and NM in a group of 172 kidney transplant patients. In a study with 810 patients, Stanulla 2005 found a lower frequency of remaining leukaemia cells above the detection limit for IM compared to NM, but not for PM on 10% of the normal 6-mercaptopurine dose compared to NM. Therefore, there are not enough indications to support an increased effectiveness for IM at the standard dose and a decrease in effectiveness with dose reduction. However, 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.
The dose adjustment calculated based on data from the literature is a reduction to 32%-100% of the standard dose (weighted mean 73%, median 46%) (n = 103). Due to the severity of the adverse event myelosuppression and the large difference between the median and weighted mean, the KNMP Pharmacogenetics Working Group decided to use the median of the calculated dose adjustment as the initial dose instead of the weighted mean. This ensures that the adverse event is avoided as far as possible, whilst dose adjustment based on toxicity and effectiveness prevents underdosing. This median was translated to 50% to be more achievable in clinical practice.
Adjustment of the initial dose should be guided by toxicity and effectiveness.
As low doses (to 1.5 mg/kg azathioprine or 0.75 mg/kg mercaptopurine per day) do not result in a significant increase in the percentage of patients with adverse events in the case of IM (Langley 2002, Jun 2005, Hindorf 2010, Eriksen 2017, and Fan 2019), dose adjustment is not required for doses up to this strength.
Because for oncology indications, it is unknown whether starting with a dose reduction based on the IM phenotype results in the same efficacy as reducing the dose based on toxicity, the KNMP Pharmacogenetics Working Group recommends 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). When treating patients with the standard dose according to acute lymphoblastic leukaemia guidelines, IM patients require a mercaptopurine dose reduction more often than NM patients (final median dose 86% for NM, 59% for *1/*3A, 63% for *1/*2, and 72% for *1/*3C (Liu 2017)). However, dose reduction is also frequently required for NM patients and there is a large overlap in the final dose range of the two phenotypes (Liu 2017).

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 7 out of the maximum of 10 points (with pre-emptive genotyping considered to be essential for scores ranging from 7 to 10 points):
Cases of unsuspected, possibly life-threatening myelosuppression have been observed in PM (code F corresponding to grade 5). The SmPC of azathioprine from the USA also reports fatal cases. This results in the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for CTCAE grade 5).
The increased risk for serious toxicity (code E corresponding to grade 4) has been shown in 3 studies and 1 systematic review (Lennard Br J Haematol 2015;169:228-40, Evans 2001, Relling 1999, and Higgs 2010). 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 study of Coenen 2015 indicates the percentage of IM+PM with leukopenia grade ≥ 2 to decrease from 22.9% to 2.6% by introducing genotype-guided dosing. However, it does not state how many of these patients have leukopenia grade ≥ 3. Because almost all PM develop severe leukopenia and intolerance on normal thiopurine doses, the prevalence of PM was used for estimation of the number needed to genotype to prevent an adverse event grade ≥ 3 instead. The frequencies of the *2-, *3A-, *3B- and *3C-alleles in the Netherlands are 0.4, 3.5, 0.4 and 0.8% respectively, corresponding to a PM frequency of 0.26%. This would amount to a number needed to genotype to find one PM, and thus one patient developing an adverse event grade ≥ 3 on normal therapy, of 384. The

calculated number needed to genotype of 384 results in 1 out of the maximum of 3 points (1 point for 100 < NNG ≤ 1000).
The Dutch Summaries of Product Characteristics (SmPCs) indicate that PM have an increased risk for myelosuppression 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).
Despite genotyping before starting azathioprine or 6-mercaptopurine scoring as essential for drug safety, results from 12 cost-effectiveness analyses are inconclusive and tend to point to a lack of cost-effectiveness (Zarca 2019 and Plumpton 2016).

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

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

<table>
<tr><th>Source</th><th>Code</th><th>Effect</th><th>Comments</th></tr>
<tr>
<td>ref. 1, imm sup
Fan X et al.
NUDT15 polymorphism confer increased susceptibility to thiopurine-induced leukopenia in patients with autoimmune hepatitis and related cirrhosis.
Front Pharmacol 2019;10:346.
PubMed PMID: 31024313.</td>
<td>3

PM: AA
IM: AA</td>
<td>149 patients with autoimmune hepatitis were treated with predniso(lo)ne for 2 weeks or till the bilirubin level fell below 6 mg/dl followed by addition of azathioprine for more than 3 months. The azathioprine dose is increased based on toxicity and response until the maintenance dose of 1-2 mg/kg is reached. The dose was reduced in patients who developed leukopenia. If any laboratory abnormality did not subside, azathioprine was discontinued. Complete blood counts were assessed weekly in the first month on azathioprine, biweekly in the following 2 months, and monthly thereafter.
Leukopenia was defined as a white blood cell count below $3x10^9$/L (or progressive deterioration for patients with leukopenia at azathioprine start (n = 13)). 8.1% of patients developed leukopenia.
Relevant co-medication was not excluded.

Genotyping:
- 145x NM
- 3x IM
- 1x PM

Results:
<table>
<tr><td colspan="3">Results for PM versus IM versus NM:</td></tr>
<tr><td></td><td></td><td>value for NM</td></tr>
<tr><td>% of patients with leukopenia</td><td>NS</td><td>7.6%</td></tr>
<tr><td colspan="3">The authors indicate that the lack of significance observed for the TPMT variant in the study is likely due to the low prevalence of TPMT variants in East Asian populations and the small sample size of the cohort.</td></tr>
</table>
NOTE: The polymorphism that is present in both NUDT15 *2 and *3 was significantly associated with leukopenia in this patient group, suggesting the lack of an association for TPMT *3C to be indeed due to the small number of patients with a variant allele. However, the maximum azathioprine dose in this study was 2 mg/kg per day instead of 1 mg/kg per day. Because the median azathioprine maintenance dose of NUDT15 IM after achieving biochemical remission was 0.96 mg/kg per day, the authors conclude that safety and efficacy</td>
<td>Authors' conclusion:
'No significant association with leukopenia was observed for TPMT*3C genotypes.'</td>
</tr>
</table>

<table>
<tr><td>ref. 1, continuation</td><td></td><td>can be maintained in most NUDT15 IM at lower azathioprine doses of approximately 1 mg/kg per day.

NOTE: The authors indicate that current guidelines, including the clinical practice guidelines for autoimmune hepatitis by the European Association for the Study of the Liver (EASL) and by the American Association for the Study of Liver Diseases (AASLD), recommend TPMT genotyping prior to initiation of azathioprine treatment (European Association for the Study of the Liver. EASL clinical practice guidelines: autoimmune hepatitis. J Hepatol 2015;63:971-1004. PubMed PMID: 26341719; Manns MP et al. Diagnosis and management of autoimmune hepatitis. Hepatology 2010:51:2193-213. PubMed PMID: 20513004).

NOTE: Genotyping was for *3C. This is the most important gene variant in this Chinese population.</td><td></td></tr>
<tr><td>ref. 2, cytostat, kinetics
Choi R et al.
Pathway genes and metabolites in thiopurine therapy in Korean children with acute lymphoblastic leukaemia.
Br J Clin Pharmacol 2019 Mar 30 [Epub ahead of print].
PubMed PMID: 30927276.</td><td>3

IM: AA</td><td>139 paediatric patients with acute lymphoblastic leukaemia were treated with maintenance therapy including 6-mercaptopurine (starting dose 50 mg/m^2 daily, median dose 30.1 mg/m^2) and methotrexate for a median period of 23.7 months. In this period, thiopurine metabolites were measured 1-14 times (median 7 times) for each individual patient). 6-Mercaptopurine and methotrexate doses were altered at the discretion of the paediatric oncologists based on the complete blood count and 6-TGN levels.
Relevant co-medication was not excluded.

Genotyping:
- 133x NM
- 6x IM (2x *1/*3C, 2x *1/*6, 1x *1/*32, 1x *1/532C)

Results:
<table><tr><td colspan="2">Dose-corrected 6-TGN concentration compared to NM (13.2 pmol per 8x10^8 red blood cells per mg/m^2):</td></tr><tr><td>IM</td><td>x 2.24 (S, but NS after correction for false discovery rate (due to multiple comparisons, i.e. multiple genes tested)</td></tr></table>
NOTE: The TPMT gene was sequenced, so genotyping was for all gene variations. A novel variant with uncertain significance (532T>C) was found in one patient.</td><td>Authors' conclusion:
'TPMT genotype was associated with thiopurine metabolism.'

Dose-corrected 6-TGN concentration versus NM:
IM: 224%</td></tr>
<tr><td>ref. 3, imm sup
Eriksen PL et al.
Enrichment of genetic variants in the glucocorticoid receptor signalling pathway in autoimmune hepatitis with failure of standard treatment.
Basic Clin Pharmacol Toxicol 2017;121:189-94.
PubMed PMID: 28374975.</td><td>3</td><td>56 patients with autoimmune hepatitis were treated with initial high-dose prednisolone, followed by tapering of prednisolone to a maintenance dose of <10 mg/day alone or in combination with azathioprine (1-2 mg/kg/day). 23 patients (41%) experienced failure of standard therapy, and other immunosuppressive regimens were applied. This group included both patients who had their treatment altered because of side effects to azathioprine and patients who did not respond to the standard regimen. The latter group also comprised one patient who had to be liver-transplanted early in the disease course.
Relevant co-medication was not excluded.

Genotyping:
- 50x NM
- 6x IM

Results:</td><td>Authors' conclusion:
'Standard treatment failure was not associated with thiopurine S-methyltransferase variants.'</td></tr>
</table>

<table>
<tr>
<td>ref. 3, continuation</td>
<td>IM: AA</td>
<td>
<table>
<tr><td colspan="2">Standard treatment failure due to adverse events or non-response compared to NM (40.0% of patients):</td></tr>
<tr><td>IM</td><td>NS</td></tr>
</table>

NOTE: Genotyping was for *2, *3A, *3B and *3C. These are the most important gene variants in this Danish population. Only *3A was identified in this patient group.
</td>
<td></td>
</tr>
<tr>
<td>ref. 4, cytostat
Liu C et al.
A genome-wide approach validates that thiopurine methyltransferase activity is a monogenic pharmacogenomic trait.
Clin Pharmacol Ther 2017;101:373-81.
PubMed PMID: 27564568.</td>
<td><u>4</u>

IM: C

(2)
PM: C</td>
<td>819 paediatric patients with acute lymphoblastic leukaemia were treated with therapy including 6-mercaptopurine.
Patients were derived from two different clinical trials. In the largest trial, 578 patients received a protocol-planned 6-mercaptopurine dose of 75 mg/m^2 per day, which was adjusted based on the degree of leukopenia and toxicities. In the smallest trial, 241 patients received 6-mercaptopurine 50-75 mg/m^2 per day, with those heterozygous for TPMT variants (13%) receiving a 6-mercaptopurine starting dose of 50-60 mg/m^2 per day. 6-Mercaptopurine dosage was also titrated based on TPMT activity and thiopurine metabolites in this trial.
To assess tolerability, dose intensity for each patient was estimated as the (total cumulative prescribed dose)/(cumulative protocol dose). P values between genotypes were determined using a general linear model that included protocol as covariate.

Genotyping:
- 745x NM
- 73x IM (6x *1/*2, 48x *1/*3A, 19x *1/*3C)
- 1x PM

Results:
<table>
<tr><td colspan="4">Median dose as percentage of the protocol dose compared to NM (86%):</td></tr>
<tr><td rowspan="3">IM</td><td>*1/*2</td><td>x 0.73</td><td rowspan="3">S for IM versus NM</td></tr>
<tr><td>*1/*3A</td><td>x 0.69</td></tr>
<tr><td>*1/*3C</td><td>x 0.84</td></tr>
<tr><td colspan="2">PM</td><td>x 0.07</td></tr>
</table>

NOTE: Genotyping was for *2, *3A and *3C. These are the most important gene variants in these patients with different genetic ancestries (largest trial) and from the USA (smallest trial).
</td>
<td>Dose versus protocol dose:
PM: 6%</td>
</tr>
<tr>
<td>ref. 5, imm sup, dose PM
van Moorsel SA et al.
Azathioprine therapy in a pediatric TPMT-deficient patient - still an option.
Ther Drug Monit 2017;39:1-4.
PubMed PMID: 28081040.</td>
<td><u>2</u></td>
<td>A 14-year old male patient with ulcerative colitis and an exacerbation on mesalazine maintenance therapy, was treated with prednisolone and azathioprine.
The authors report the therapeutic range of 6-TGN to be 235-490 pmol/8x10^8 red blood cells (RBC). This therapeutic range is dependent on the method of measurement.

Results:
<table>
<tr><td>- Three weeks after the start of azathioprine 175 mg (2.5 mg/kg) once daily, the 6-TGN levels were 4.3 times the upper limit of the therapeutic range (2095 pmol/8x10^8 RBC). 6-methylmercaptopurine metabolites were not detectable. Leukocyte and platelet count showed no signs of myelotoxicity (9.9x10^9/L and 311x10^9/L, respectively).</td></tr>
<tr><td>- Two weeks after a 71% reduction of the azathioprine dose to 50 mg (0.71 mg/kg) once daily, the 6-TGN levels were increased to 4.8 times the upper limit of the therapeutic range (2353 pmol/8x10^8 RBC). Azathioprine treat-</td></tr>
</table>
</td>
<td>Authors' conclusion:
'We demonstrate that azathioprine therapy still might be an effective and safe therapeutic option in pediatric thiopurine S-methyltransferase-deficient inflammatory bowel disease patients.'</td>
</tr>
</table>

ref. 5, continuation	PM: B	ment was stopped. After 3 weeks, a mild thrombocytopenia ($101x10^9$/L) was shown, which resolved spontaneously within 1 week. Genotyping showed the patient to be PM (*3A/*3C). - After 6.5 weeks azathioprine was restarted in a dose of 75 mg once weekly (corresponding to 0.15 mg/kg per day). 6-TGN levels increased from 0.66 times to 1.23 times the upper limit of the therapeutic range (321 to 605 pmol/$8x10^8$ RBC) in 3 weeks. - After a reduction of the azathioprine dose to 50 mg once weekly (corresponding to 0.10 mg/kg per day, i.e. 4% of the normal dose), the 6-TGN levels remained between 500 and 600 pmol/$8x10^8$ RBC without signs of myelotoxicity. After 11 weeks, leukocyte count showed a mild leukopenia ($3.0\text{-}4.0x10^9$/L), which recovered within 5 weeks without intervention. Platelet count was normal (between 175 and $271x10^9$/L). The patient was in clinical remission on a maintenance dose of 50 mg azathioprine once weekly for almost 5 years at the time of reporting. NOTE: The authors indicate that pre-emptive TPMT testing is suggested by guidelines of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (Turner D et al. Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines. J Pediatr Gastroenterol Nutr 2012;55:340-61. PubMed PMID: 22773060).Therefore, all patients with inflammatory bowel disease in their hospital currently undergo pre-emptive TPMT testing when thiopurine therapy is indicated.	Dose versus normal dose: PM: 4%
ref. 6, imm sup Coenen MJ et al. Identification of patients with variants in TPMT and dose reduction reduces hematologic events during thiopurine treatment of inflammatory bowel disease. Gastroenterology 2015;149:907-17. PubMed PMID: 26072396.	3	783 patients with inflammatory bowel disease were treated with azathioprine (64% of patients) or 6-mercaptopurine (36% of patients). Follow-up was for a period of 20 weeks. Genotype-guided treatment (n = 405) was compared to standard treatment (n = 378). Standard treatment was azathioprine 2-2.5 mg/kg/day or 6-mercaptopurine 1-1.5 mg/kg/day). In the genotype-guided group, NM received the normal thiopurine dose and IM 50% of the normal dose. PM were scheduled to receive 0-10% of the normal dose and the only PM in the study did not receive a thiopurine. 13% of patients in the standard treatment group and 15% of patients in the genotype-guided group did not receive the allocated intervention, mostly (for 84% and 90% respectively) due to a starting dose not according to the advice. Gastroenterologists were allowed to change the thiopurine dose or stop treatment when a side effect occurred. The guidelines were to consider a dose reduction by a leukocyte count ≤ $4x10^9$/L and a fast decrease of leukocyte count, to reduce the dose with 50% by a leukocyte count of ≤ $3x10^9$/L, and to stop treatment by a leukocyte count < $1x10^9$/L. Treatment re-challenge was at the discretion of the gastroenterologist. Two patients died due to infections, one IM who was started on a reduced thiopurine dose and one NM. Hematologic adverse events were defined as leukocyte count < $3.0x10^9$/L or platelet count < $100x10^9$/L. Disease activity was based on the Harvey-Bradshaw Index for Crohn's disease (n = 356) or the partial Mayo score for ulcerative colitis (n = 253). Remission was defined as a score on the Harvey-Bradshaw Index < 5 (< 26.3% of the maximum score) and a partial Mayo score < 3 (< 33.3% of the maximum score). General adverse events included dizziness, shivers, fever, and general malaise. Gastrointestinal adverse events inclu-	Authors' conclusion: 'Screening for variants in TPMT did not reduce the proportions of patients with hematologic adverse drug reactions (ADRs) during thiopurine treatment for IBD. However, there was a 10-fold reduction in hematologic ADRs among variant carriers who were identified and received a dose reduction, compared with variant carriers who did not, without differences in treatment efficacy.'

<table>
<tr>
<td>ref. 6, continuation</td>
<td>Genotype-guided versus standard treatment: all: AA IM+PM: AA#</td>
<td>
ded stomach ache, diarrhoea, reduced appetite, nausea, and vomiting. Hepatic adverse events included cholestasis, cholangitis, hepatitis, and steatosis. Dermatological adverse events included hair loss, warts, and skin rash.
Co-treatment with allopurinol was excluded, but 49.6% of patients used mesalazine concomitantly. In addition, corticosteroids (81.7% of patients) and biologicals were used as co-medication. The use of mesalazine and corticosteroids did not differ significantly between the genotype-guided and standard treatment group, but the use of biologicals was higher in the standard treatment group (7.4% versus 3.7%). The use of biologics was associated with an increased risk of hematologic adverse events.
The study was designed to have 80% power with inclusion of 388 patients per treatment arm and a reduction in hematologic adverse event rate of 50% (event rate of 11% in the non-genotyped group and 5.5% in the genotyped group).

Genotyping:
Genotype-guided group
- 365x NM
- 39x IM
- 1x PM

Standard treatment group
- 340x NM
- 38x IM

Results:
<table>
<tr><td colspan="4">Results compared to the standard treatment group (controls):</td></tr>
<tr><td colspan="2"></td><td></td><td>value for controls</td></tr>
<tr><td colspan="2">% of patients with hematologic adverse events</td><td>NS</td><td>7.9%</td></tr>
<tr><td colspan="2"></td><td>The result was also NS when:
- patients on biologicals were excluded
- only patients who started treatment were included
- the median time to a hematologic adverse event was analysed
- only events in the first 8 weeks were analysed</td><td></td></tr>
<tr><td colspan="2"></td><td>The authors calculated that a randomized controlled trial with 42,556 participants would be needed to show a benefit for the entire intervention group (power of 80%).</td><td></td></tr>
<tr><td rowspan="3">% of patients who started treatment, with hematologic adverse events</td><td>NM</td><td>NS</td><td>6.6%</td></tr>
<tr><td rowspan="2">IM+PM</td><td>RR = 0.11 (95% CI: 0.01-0.85)</td><td rowspan="2">22.9%</td></tr>
<tr><td>The result was</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

ref. 6, continuation				also S when patients on biologicals were excluded.	
		% of patients with treatment induced remission		NS	67.4%
		median absolute change in erythrocyte sedimentation rate	NM	NS	-1.0
			IM+PM	- 8.0 (S)	0.0
		median percentage change in erythrocyte sedimentation rate	NM	NS	-6.3%
			IM+PM	NS	0.0%
		median absolute change in C-reactive protein		NS	-1.0
		median percentage change in C-reactive protein		NS	-22.9%
		% of patients with general adverse events		NS	43.1%
		% of patients with gastrointestinal adverse events		NS	71.2%
		% of patients with infections		NS	4.5%
		% of patients with hepatic adverse events		NS	7.1%
		% of patients with dermatologic adverse events		NS	23.3%
		% of patients with myalgia		NS	13.8%
		% of patients with hematologic adverse events		NS	16.4%
		% of patients with thiopurine use for up to 20 weeks		NS	69.3%
		% of patients with (temporary) thiopurine stop		NS	37.8%
		% of patients with signs of hepatotoxicity, pancreatitis or anemia based on blood levels		NS	25.9%, 22.2%, and 61.1%
		azathioprine starting dose	all	NS	2.2 mg/kg
			NM	NS	2.2 mg/kg
			IM+PM	x 0.52 (S)	2.1 mg/kg
		6-mercaptopurine starting dose	all	x 1.0 (S)	1.2 mg/kg
			NM	NS	1.2 mg/kg
			IM	x 0.50 (S)	1.2 mg/kg
		azathioprine dose in week 20	all	x 0.95 (S)	2.2 mg/kg
			NM	NS	2.2 mg/kg
			IM+PM	x 0.48 (S)	2.1 mg/kg
		6-mercaptopurine dose in week 20	all	NS	1.1 mg/kg
			NM	NS	1.1 mg/kg
			IM	x 0.55 (S)	1.1 mg/kg
		The median 6-TGN level after 8 weeks was within the therapeutic range for IM+PM on genotype-guided treatment and above therapeutic range for IM on standard treatment. The 6-TGN level was significantly different between the two groups. For NM, median 6-TGN levels			

ref. 6, continuation		after 8 weeks were around the lower limit of the therapeutic range for both genotype-guided and standard treatment. NOTE: Genotyping was for *2, *3A and *3C. These are the most important gene variants in this Dutch population.	
ref. 7, cytostat Lennard L et al. Thiopurine methyltransferase and treatment outcome in the UK acute lymphoblastic leukaemia trial ALL2003. Br J Haematol 2015;170:550-8. PubMed PMID: 25940902.	3	2387 patients, aged 1-25 years, with acute lymphoblastic leukaemia were treated with therapy including 6-mercaptopurine. The trial recommendation was to start PM on 10% of the 6-mercaptopurine protocol dose, and titrate to the protocol target cell counts. Median follow-up was 5 years 10 months (range 3 months to 10 years 1 month). 3% of patients had metabolite levels at the lower limit of detection or lacked measurable metabolites, suggesting non-compliance, 15% of these patients on multiple occasions. Patients classified as clinical high risk (NCI re-classified cohorts, high-risk cytogenetics or slow morphological early response) were not eligible for minimal residual disease (MRD) stratification. For the stratification of clinical standard and intermediate risk groups by bone-marrow minimal residual disease (MRD), MRD was measured after induction (day 29) and again after the recovery from consolidation but prior to the start of interim maintenance. Minimal residual disease low-risk patients were defined as those with no detectable disease and those patients who were MRD negative prior to interim maintenance. Indeterminate risk patients had detectable disease (<0.01% MRD = $<10^{-4}$ leukaemia cells) prior to interim maintenance; this group also included those patients with no MRD measurement. High risk patients had detectable disease (≥0.01%) at the end of induction. TPMT genotypes were available for 791 high-risk MRD patients and 866 low-risk MRD patients. Treatment intensity randomizations of one or two delayed intensive blocks (reduced versus standard treatment) for low risk patients and standard treatment versus an intensive schedule for high-risk patients was applied. The delayed intensive blocks did not contain 6-mercaptopurine. Consolidation therapy for all patients, interim maintenance courses for clinical standard and intermediate risk patients, and maintenance therapy for all patients contained 6-mercaptopurine. Additional blood samples for metabolite monitoring were taken if non-compliance with oral 6-mercaptopurine was suspected or for patients unduly sensitive to mercaptopurine or tolerating mercaptopurine prior to dose escalation. Thiopurine metabolites were measured after a median of 17 weeks in 54% of NM, 58% of IM and all PM. For the 7 PM identified before treatment, thiopurine metabolites were measured after a median of 8 weeks (range 2 to > 12 weeks). Comparing the 2406 patients with thiopurine data (TPMT genotype and/or mercaptopurine metabolites) to 720 patients from the same trial with no data, there is some bias in the thiopurine dataset towards younger patients and those who are less high risk. Event-free survival (EFS) was defined as time to relapse, secondary tumour or death. Relapse-free survival (RFS) was defined as time to relapse (excluding those patients who did not achieve a remission or died during initial induction or consolidation chemotherapy). Overall survival (OS) was defined as time to death. Comparisons between groups were stratified by age, gender and white blood cell count at presentation. To test the hypothesis that there is about a four-fold differen-	Authors' conclusion: 'In contrast to the preceding trial ALL97, there was no difference in event-free survival between the TPMT genotypes. ….. In conclusion, refinements in risk stratification and treatment have reduced the influence of TPMT genotype on treatment outcome in a contemporary protocol.'

ref. 7, continuation

ce in event rates between TPMT *1/*3A and *1/*3C groups, as seen in Lennard, Br J Haematol 2015;169:228-40, 1845 patients over a six-year trial period will give over 95% power to detect this with similar event rates (55% and 14% for TPMT*1/*3C and TPMT*1/*3A patients respectively). The event-free survival in this study is higher than in Lennard, Br J Haematol 2015;169:228-40. There is over 85% power to detect a similar difference but with decreased event rates of 40% and 10%, and over 80% for 32% and 8% for TPMT *1/*3C and TPMT*1/*3A patients, respectively.

Genotyping:
- 2190x NM
- 189x IM (3x *1/*2, 166x *1/*3A, 19x *1/*3C, 1x *1/*9)
- 8x PM (of whom 7 identified pre-treatment)

Results:

Results compared to NM (PM versus IM versus NM, unless indicated otherwise):			
			value for NM
% of patients with 5-years event-free survival		NS	88%
		The result was also NS: - when separate genotypes were compared to NM - within both the MRD high-risk and MRD low-risk group	MRD high-risk: 80.5% MRD low-risk: 95.4%
% of patients with 5-years relapse-free survival		NS	92%
% of patients with 5-years overall survival		NS	93%
median 6-mercaptopurine dose at time of metabolite measurement	IM	x 0.987 (S for the difference)	75 mg/m^2
median 6-TGN	IM	x 2.41 (S for the difference)	312 pmol/8 x10^8 RBC
median 6-methyl-mercaptopurines nucleotides (6-MMPN)	IM	x 0.28 (S for the difference)	14808 pmol/8 x10^8 RBC
median maximum tolerated 6-mercaptopurine dose	PM	x 0.12 (range: x 0.11 - x 0.35)	Protocol: 75 mg/m^2
median 6-TGN at maximum tolerated 6-mercaptopurine dose	PM	x 4.26 (range: x 3.11 - x 8.23)	312 pmol/8 x10^8 RBC
One PM, who lacked a pre-treatment blood sample, was identified during maintenance chemotherapy with a history of repeated cytopenias and an inability to tolerate mercaptopurine; 6-TGN level after 67% of protocol 6-mercaptopurine dose (50 mg/m^2) for 6 weeks was 7.52 times the median value for NM (2347 pmol/8x10^8 RBC).			

IM: A

PM: A

(2)
PM: E

Median dose versus protocol dose:
PM: 12%

Dose-corrected 6-TGN concentration versus NM:
PM: 1128%

ref. 7, continuation		NOTE: The TPMT activity was measured in 48% of NM and 49% of IM. The median mercaptopurine metabolite concentrations measured in the NMs with a TPMT activity comparable to the IMs (6-TGN: 317 pmol/8x10^8 RBC, 6-MMPN: 15,937 pmol/8x10^8 RBC) were similar to the concentrations measured in the NMs with a TPMT activity higher than the IMs (6-TGN: 311 pmol/8x10^8 RBC, 6-MMPN: 14,380 pmol/ 8x10^8 RBC) and significantly different from the metabolite concentrations recorded for IMs (6-TGN: 747 pmol, 6-MMPN: 3407 pmol) (S). This has been mainly attributed to the undue influence of the disease process and chemotherapy on red blood cell TPMT enzyme activity. In this patient group, genotyping provides more information than phenotyping. NOTE: Genotyping was by sequencing exons 5, 7 and 10, detecting *2, *3A, *3B, *3C and *9. These are the most important gene variants in this British population.	
ref. 8 - cytostat Lennard L et al. Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: the influence of thiopurine methyltransferase pharmacogenetics. Br J Haematol 2015;169:228-40. PubMed PMID: 25441457.	3 IM: E	A total of 709 children with acute lymphoblastic leukaemia were treated with mercaptopurine for 2-3 years. The initial dose was 75 mg/m^2 for NM and IM, and 7.5 mg/m^2 for PM. Mercaptopurine was administered in combination with methotrexate, vincristine and either dexamethasone or prednisone. Relevant co-medication was not excluded. Clinical outcome measures were only determined in combination with a group receiving thioguanine as the thiopurine (n = 426) and were available for 61% of the patients. A dose of 100% was defined as the initial dose of the thiopurine for NM/IM. Genotyping (mercaptopurine only): - 636x NM (*1/*1) - 71x IM (3x *1/*2, 53x *1/*3A, 12x *1/*3C, 1x *1/*9, 1x *1/*32, 1x *1/*33) - 2x PM (1x*2/*3A, 1x *3C/*3C) IM versus NM: Mercaptopurine or thioguanine: - duration of cytopenia-induced thiopurine dose interruptions increased by 34% (from 15.5% to 20.8% of the total duration) (S) - neutropenia increased by 8.1% (from 23.4% to 25.3% of the total duration) (S) - thrombocytopenia increased by 159% (from 3.4% to 8.8% of the total duration) (S) - the average daily thiopurine dose decreased by 10% (from 78.0% to 70.4% of the initial dose) (S) - 5-year EFS (event-free survival, with an event defined as time to relapse or death) increased by 10% for *1/*3A versus NM (from 80% to 88%) (S), but multivariate regression analysis did not identify a significantly decreased risk of relapse or death for all IM patients except for those with *1/*3C (NS) - 5-year EFS decreased by 34% in *1/*3C patients versus NM patients (from 80% to 53%) (S), and multivariate regression analysis showed an increased risk of relapse or death (HR = 3.2; 95% CI: 1.5-6.8) (S) There was no difference between *1/*3C and *1/*3A in average daily dose or incidence of cytopenia. However, there was evidence of poor compliance in the mercaptopurine group (see below). - no difference in secondary tumours (median follow-up 11.3 years) (NS)	Authors' conclusion: "TPMT*1/*3A heterozygotes had a better event-free survival than TPMT wild-type patients. Thiopurine induced cytopenias were not detrimental to treatment outcome. .... The TPMT heterozygotes tolerated significantly lower average % doses than the TPMT wild-type patients (70% vs 78% for TPMT wild-type, a daily-dose difference of 6 mg/m^2 per day mercaptopurine). However, the range of thiopurine doses tolerated was wide, with the upper and lower limits similar for both TPMT genotypes. These findings do not support any change in the prescribing criteria (both genotypes start at the same standard protocol dose and titrate to toxicity)."

ref. 8, continuation	PM: A (2)	Mercaptopurine only: - Increase in the median 6-TGN concentration by 109% (from 360 to 754 pmol/8x10^8 RBCs) (S) measured at a non-significantly different median dose (from 75 to 74 mg/m^2) (NS) - the median 6-TGN concentration was higher for *1/*3A than for *1/*3C, despite similar doses and TPMT activity (802 and 608 pmol/8x10^8 RBCs; increase versus NM of 123% and 69%) (S). There was also a trend for lower concentrations of the metabolite MMP for *1/*3C, suggesting that the lower 6-TGN concentrations are caused by a lower therapy compliance. PM versus NM: Mercaptopurine only: - The eventual dose for *2/*3A was 5% of the dose in NM patients (7.5 mg/m^2 every other day) and 20% (15 mg/m^2) for *3C/*3C. - At these doses, the 6-TGN concentrations were a factor 4.6 and 5.0 higher, respectively, than the median 6-TGN concentration for NM (1670, 1784 and 360 pmol/8x10^8 RBCs respectively). NM on mercaptopurine or thioguanine: - The average daily thiopurine dose for *2/*3A was 16% of the dose in NM patients (12.6% of the initial dose for NM/IM). - The average daily thiopurine dose for *3C/*3C was 25% of the dose in NM patients (19.5% of the initial dose). N.B.: Genotyping was performed for *2, *3A, *3B and *3C. Exons 3 to 10 were sequenced to identify new or rare variants (*9, *21, *32-*34).	Dose versus NM: PM: 12.5%
ref. 9 - cytostat, dose PM Belen BF et al. Severe myelotoxicity associated with thiopurine S-methyltransferase*3A/*3C polymorphisms in a patient with pediatric leukemia and the effect of steroid therapy. Turk J Haematol 2014;31:399-402. PubMed PMID: 25541649.	2 PM: E	A fifteen year old girl with acute lymphoblastic leukaemia developed two prolonged episodes of myelosuppression shortly after starting chemotherapy with mercaptopurine 60 mg/m^2 per day, cytarabine and cyclophosphamide. Despite the use of colony stimulating factors, she developed neutropenia (< 0.8x10^9 cells/mm^3) on maintenance therapy with mercaptopurine and methotrexate at doses amounting to 25% of the doses stated in the protocol. Her TPMT genotype was *3A/*3C. In addition, she was heterozygous for the MTHFR polymorphisms C677T and A1298C. For the MTHFR polymorphisms, it is not clear whether they form an additional risk factor for haematotoxicity. Pancytopenia and transfusion-dependency continued after reduction of the doses of mercaptopurine and methotrexate to 10% of the doses listed in the protocol. Intensification therapy with high-dose methotrexate and mercaptopurine at 5% of the standard dose (2.5 mg/m^2 per day) was possible with weekly transfusions to keep the blood platelets above 10x10^9/L. Maintenance therapy over a period of 5 weeks was possible at 5-10% of the standard dose of mercaptopurine and 8-16% of the standard dose of methotrexate.	Authors' conclusion: "Compound heterozygosity for TPMT *3A/3C may be associated with severe bone marrow hypoplasia, even with minimal amounts of MP, in children with ALL." Dose versus NM: PM: 7.5%
ref. 10 - imm sup, kinetics Kim MJ et al. Monitoring thiopurine metabolites in Korean pediatric	3	109 children and adolescents with inflammatory bowel disease were treated with azathioprine. Relevant co-medication was not excluded. Genotyping: - 102x NM (*1/*1)	Authors' conclusion: "There were no statistical differences in initial AZA dose between the

patients with inflammatory bowel disease. Yonsei Med J 2014;55:1289-96. PubMed PMID: 25048487. **ref. 10, continuation**	IM: AA PM: A (2)	- 6x IM (4x *1/*3C, 1x *1/*6, 1x *1/*16) - 1x PM (*3C/*3C) IM versus NM: - dose-corrected 6-TGN concentration increased by 183% (from 347.3% to 983.0 pmol/8x10^8 RBC per mg/kg per day) (NS) PM versus NM: - dose-corrected 6-TGN concentration increased by 598% (from 347.3% to 2425.6 pmol/8x10^8 RBC per mg/kg per day) For all TPMT genotypes, the required dose was lower for East Asians than for Western patients. N.B.: The TPMT gene was sequenced for the identification of variants. *3C is the most common gene variant in this East Asian population group.	group of wild type TPMT and TPMT mutation. However, the 6-TGN concentration was 416.8±271.7 pmol/8×10^8 RBC in patients with wild type TPMT and 1822.9± 1493.9 pmol/8×10^8 RBC in TPMT mutation (p=0.001)." Dose-corrected 6-TGN concentration versus NM: IM: 283% PM: 698%
ref. 11 - cytostat Levinsen M et al. Pharmacogenetically based dosing of thiopurines in childhood acute lymphoblastic leukemia: influence on cure rates and risk of second cancer. Pediatr Blood Cancer 2014;61:797-802. PubMed PMID: 24395436.	3 IM with 67% of the standard initial dose: AA	A total of 674 children with acute lymphoblastic leukaemia were treated with mercaptopurine for 2 or 2.5 years. The initial dose was 75 mg/m^2 for NM, 50 mg/m^2 for IM and 5-10 mg/m^2 for PM. Mercaptopurine was administered in combination with methotrexate, vincristine and dexamethasone. The duration of the mercaptopurine treatment and the additional cytostatic treatments was dependent on the risk group. Relevant co-medication was not excluded. Data were compared to those from a study in which IM received an initial dose of 75 mg/m^2 (n = 601, of which 75 with an IM or PM phenotype or genotype). Genotyping: - 617x NM - 56x IM - 1x PM IM+PM with reduced initial dose compared to NM with standard initial dose: - no difference in EFS at 8 years (NS) - no difference in the 8-year risk of developing a new cancer (NS) - no difference in the 8-year risk of cancer relapse (NS). The same result was observed after correction for confounding factors in Cox regression analysis. IM+PM with reduced initial dose compared to phenotypically or genotypically IM+PM with standard initial dose: - decrease in the number of patients who developed a new cancer by 100% (from 4 to 0), following exclusion of 2 IM who received a standard initial dose after all (S). In the total group (including the IM who received the standard initial dose after all and developed a new cancer), there was no significant difference in the 8-year risk of developing a new cancer (NS). - increase in the 8-year risk of cancer relapse by a factor 2.9 (from 6.7% to 19.7%) (S) N.B.: Genotyping was performed for *3A, *3B and *3C.	Authors' conclusion: "This study indicates that reducing 6MP starting dose for patients with $TPMT^{LA}$ may reduce second malignant neoplasma risk but lead to a relapse risk similar to that of patients with $TPMT^{WT}$. ..... Given the low relapse risk for patients with $TPMT^{LA}$ receiving starting 6MP doses of 75 mg/m^2 in NOPHO ALL92, the present study suggests that patients with $TPMT^{LA}$ or $TPMT^{WT}$ both should be treated with starting doses of 75 mg 6MP/m^2/day. Since longer duration of therapy has been associated with second malignant neoplasm, one option could be to shorten the duration of maintenance therapy for patients with $TPMT^{LA}$ to 2 years as given in BFM protocols."
ref. 12 - cytostat, dose PM Demlova R et al.	2	A thirteen year old boy with acute lymphoblastic leukaemia developed very severe myelosuppression with recurrent cerebral haemorrhages upon treatment with standard doses	Authors' conclusion: "Extreme and life-

Augmenting clinical interpretability of thiopurine methyltransferase laboratory evaluation. Oncology 2014;86:152-8. PubMed PMID: 24643197.	PM: E	of mercaptopurine, cytarabine and cyclophosphamide. His genotype turned out to be *2/*3A. The patient was treated successfully with 6.5% of the standard dose of mercaptopurine.	threatening toxicity was observed in the compound heterozygote patient." Dose versus NM: PM: 6.5%
ref. 13 – imm supp, dose PM Lee MN et al. Successful azathioprine treatment with metabolite monitoring in a pediatric inflammatory bowel disease patient homozygous for TPMT*3C. Yonsei Med J 2013;54:1545-9. PubMed PMID: 24142665.	2 PM: E	An eighteen year old male with Crohn's disease developed neutropenia ($1.0x10^9$/L) and leukopenia ($2.8x10^9$/L) two weeks after starting a standard dose of azathioprine (1.8 mg/kg per day) and mesalazine 55.6 mg/kg per day. Despite reduction of the azathioprine dose to 0.9 mg/kg per day, the neutropenia and leukopenia had become worse three weeks later ($0.19x10^9$/L and $1.9x10^9$/L respectively). After starting again with azathioprine (0.8 mg/kg per day), without mesalazine, the patient again developed neutropenia after the dose was increased to 1.2 mg/kg per day. The patient was found to have the *3C/*3C genotype. Based on 6-TGN concentrations, the patient was given azathioprine 0.2 mg/kg per day for 1.5 years and then 0.1 mg/kg per day for 0.5 years without further episodes of neutropenia or leukopenia.	Dose versus NM: PM: 8.3%
ref. 14 - cytostat, dose PM Kim H et al. Pharmacogenetic analysis of pediatric patients with acute lymphoblastic leukemia: a possible association between survival rate and ITPA polymorphism. PLoS One 2012;7:e45558. PubMed PMID: 23029095.	3 PM: A (2)	Out of a total of 100 children with acute lymphoblastic leukaemia who received maintenance therapy with mercaptopurine and methotrexate, 93 were NM and 1 was PM (*2/*2). The planned dose was 50 mg/m^2 per day. The required dose is lower for East Asians than for Western patients. Relevant comedication was not excluded. PM versus NM: - the PM exhibited only mild toxicity - the dose in the last cycle of the maintenance therapy was 62% for PM and median 50% for NM (increase by 24%) N.B.: Genotyping was performed for *2, *3A, *3B and *3C. In this Asian population group, *3C was the most common gene variant.	Dose versus NM: PM: 124%
ref. 15 - imm sup Booth RA et al. Assessment of thiopurine S-methyltransferase activity in patients prescribed thiopurines: a systematic review. Ann Intern Med 2011;154:814-23, W-295-8. PubMed PMID: 21690596.	4 IM: C	Meta-analysis of 31 studies into toxicity caused by azathioprine or mercaptopurine in a total of 3,638 patients with autoimmune diseases (including 260 IM and 19 PM). Leukopenia was the measure of outcome in 18 studies involving a total of 1,825 patients, including 105 IM and 7 PM. Of these 18 studies, Jun, 2005 and Zelinkova, 2006 have also been included separately in this risk analysis. Only studies in which at least *2, *3A, *3B and *3C were genotyped were included in the meta-analysis. IM versus NM: - increased risk of leukopenia: OR = 4.29 (95% CI: 2.67-6.89) (S) - increase in therapy withdrawal due to adverse events: OR = 6.54 (95% CI: 2.53-16.91) (4 studies with 27 IM) (S) - no difference in the risk of other adverse events (infections, myelotoxicity, anaemia, thrombocytopenia, hepatotoxicity and pancreatitis) (NS). The total number of patients in the studies into these adverse events was lower. PM versus NM:	Authors' conclusion: "Compared with non-carriers, heterozygous and homozygous genotypes were both associated with leukopenia."

<table>
<tr><td>ref. 15, continuation</td><td>PM: C</td><td>- increased risk of leukopenia: OR = 20.84 (95% CI: 3.42-126.89) (5 studies with 7 PM) (S)
- no difference in the risk of other adverse events (myelotoxicity, hepatotoxicity and pancreatitis) (NS).
The total number of patients in the studies into these adverse events was lower.</td><td></td></tr>
<tr><td>ref. 16 - imm sup
Newman WG et al. A pragmatic randomized controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study. Pharmacogenomics 2011;12:815-26. PubMed PMID: 21692613.</td><td>3

Genotype-guided</td><td>333 patients with inflammatory diseases were treated with azathioprine. Follow-up was for a period of 4 months. Genotype-guided treatment (n = 167) was compared to standard treatment (n = 166). Clinicians were advised to start with a maintenance dose of azathioprine (i.e., 1.5-3 mg/kg/day) for NM; to start azathioprine at a low dose (i.e., 25-50 mg/day) and titrate to the maintenance dose for IM; and not to start azathioprine, but to use an alternative treatment for PM.
13 patients never started azathioprine. Of the 322 patients with data available at 4 months (163 in the genotype-guided and 159 in the standard treatment group), 28.3% had stopped azathioprine due to adverse drug reactions. Nausea and vomiting was the most common adverse drug reaction (16% of patients), followed by hepatotoxicity (8.4% of patients), malaise (7.1% of patients) and myalgia (6.8% of patients). Hepatotoxicity was defined as alanine transaminase ≥ two times the upper limit of the normal range. Severe neutropenia was defined as <1.0x10^9/l and moderate neutropenia as 1.0-1.5x10^9/l.
Disease severity after 4 months was known for 112 Crohn's disease patients and was measured with the Harvey Bradshaw Index. A score > 5 indicates active disease. Disease activity significantly decreased during treatment.
Co-treatment with allopurinol was excluded, but co-treatment with mesalazine (32% of patients) and other medication contributing to adverse drug reactions and immunosuppression was not. There were no significant differences in co-medication between the genotype-guided and standard treatment group.
The study was originally designed to have 80% power, to detect a change in the incidence of severe haematological adverse drug reactions that required the dose of azathioprine to be reduced or the treatment to be stopped in the first 4 months of therapy from 14 to 8%. This required 500 patients in each arm. However, because neutropenia rates in the first 100 patients were considerably lower than initially predicted (1.3%), the study was resized to have 80% power to detect a 40% reduction in stopping azathioprine due to occurrence of an adverse drug reaction in the first 4 months of treatment. This required a total of 330 patients.

Genotyping:
Genotype-guided group
- 148x NM
- 19x IM

Standard treatment group
- 150x NM
- 15x IM
- 1x PM

Results:
Results compared to the standard treatment group (controls):
<table><tr><td></td><td></td><td>value for controls</td></tr><tr><td>% of patients stopping azathioprine due to adverse events</td><td>NS</td><td>27.7%</td></tr></table></td><td>Authors' conclusion:
'Our work supports the strong evidence that individuals with TPMT variant homozygosity are at high risk of severe neutropenia, whereas TPMT heterozygotes are not at increased risk of adverse drug reactions at standard doses of azathioprine.'</td></tr>
</table>

ref. 16, continuation	versus standard treatment: AA (2) PM: D	% of patients with any adverse event		NS	32.1%	
		% of patients with hepatotoxicity		x 2.3 (S, but NS after correction for multiple comparisons) Hepatotoxicity was only observed in NM.	5.0%	
		% of patients with severe neutropenia		NS The only patient with severe neutropenia was the only PM. This patient experienced severe, early-onset nonfatal neutropenia after start of standard treatment (starting dose 0.6 mg/kg per day).	0.6%	
		% of patients with moderate neutropenia		NS	0.0%	
		prevalence of each of the other tested adverse events		NS		
		Crohn's disease severity after 4 months (score on the Harvey Bradshaw Index)		NS	4.5	
		azathioprine starting dose	NM	NS	0.86 mg/kg	
			IM+PM	x 0.66 (S)	0.93 mg/kg	
		azathioprine dose at 4 months	NM	NS	1.74 mg/kg	
			IM	NS	1.62 mg/kg	

Results for IM (on genotype-guided or standard treatment) compared to NM:		
		value for NM
% of patients stopping azathioprine due to adverse events	NS	28.2%
% of patients with moderate or severe neutropenia	NS The result was also NS when follow-up of patients who were still taking azathioprine at 4 months was extended to 12 months.	0.70%

NOTE: The authors indicate that a recommendation for TPMT testing has been added to clinical guidelines of the British Association of Dermatology and the British Society for Rheumatology/British Health Professionals in Rheumatology Standards (Anstey AV et al. Guidelines for prescribing azathioprine in dermatology. Br J Dermatol 2004;151:1123-32. PubMed PMID: 15606506; and Chakravarty K et al. BSR/BHPR guideline for disease-modifying anti-rheumatic drug (DMARD) therapy in consultation with the British Association of Dermatologists. Rheumatology (Oxford) 2008;47:

ref. 16, continuation		924-5. PubMed PMID: 16940305). NOTE: Genotyping was for *2, *3A, *3B and *3C. These are the most important gene variants in this British population. No rare TPMT variant alleles were identified in any patient by a screen of all previously reported TPMT variants.	
ref. 17 - imm sup Dong XW et al. Thiopurine S-methyltransferase polymorphisms and thiopurine toxicity in treatment of inflammatory bowel disease. World J Gastroenterol 2010;16:3187-95.	3 (IM+PM) : C	Meta-analysis of the data from 9 studies, including Zelinkova 2006, with a total of 1,309 patients with Crohn's disease or ulcerative colitis on azathioprine 1.49-2.5 mg/kg per day or mercaptopurine 0.71-1.25 mg/kg per day. (IM+PM) versus NM: - higher risk of adverse events: OR = 2.93 (CI: 1.68-5.09) - higher risk of adverse events other than hepatotoxicity and pancreatitis: OR = 4.37 (CI: 1.69-11.29) - higher risk of bone marrow toxicity: 20.9% versus 4.5%; OR = 5.93 (CI: 2.96-11.88) - no increased risk of hepatotoxicity (OR = 1.51 (NS)) and pancreatitis (OR = 1.02 (NS)) Similar results were obtained if studies with a different definition of the adverse event were excluded one by one, if studies with dose AZA > 2 mg/kg or 6-MP > 1 mg/kg per day were excluded, if 8 studies that did not meet all the inclusion criteria were included in the analysis and if only studies with ≥ 100 patients were included.	Authors' conclusion: "This meta-analysis suggests that the TPMT polymorphisms are associated with thiopurine-induced overall ADRs and BMT, but not with hepatotoxicity and pancreatitis."
ref. 18 - imm sup Hindorf U et al. Characterisation and utility of thiopurine methyltransferase and thiopurine metabolite measurements in autoimmune hepatitis. J Hepatol 2010;52:106-11.	3 IM: A	Thiopurine therapy was not withdrawn in 143 patients (134x azathioprine, 9x mercaptopurine) out of 175 patients with auto-immune hepatitis (156x NM, 12x IM, 1x genotypic NM/ phenotypic IM, 2x genotypic IM/phenotypic NM, 5 unknown genotype). Dose and 6-TGN concentration were determined for these 143 patients. Co-medication: prednisolone, otherwise unknown. Phenotypically IM versus phenotypically NM: - decrease in dose by 15% (from 1.3 to 1.1 mg/kg per day) (NS). - increase in 6-TGN concentration by 84% (from 112 to 206 pmol/8x10^8 RBC) (S) - no increase in the percentage of patients with adverse events (from 16% to 20%) (NS). N.B.: Lower doses are used in the case of auto-immune hepatitis than for Crohn's disease and ulcerative colitis (approx. half), meaning that bone marrow toxicity plays a less important role here.	Dose versus NM (corrected for 6-TGN concentrations): IM: 46%
ref. 19 - imm sup Sheffield LJ et al. Thiopurine methyltransferase and thiopurine metabolite testing in patients with inflammatory bowel disease who are taking thiopurine drugs. Pharmacogenomics 2009;10:1091-9.	3 IM: A	A total of 126 patients with Crohn's disease or ulcerative colitis (113x NM, 13x IM), who used thiopurines ≥ 3 months, of which ≥ 4 weeks at a stable dose. No co-medication reported. IM versus NM: - decrease in dose by 22% (from 2.02 to 1.58 mg/kg azathioprine-equivalents per day) (S) - increase in 6-TGN concentration by 83% (from 341.5 to 624.9 pmol/8x10^8 RBC) (S) N.B.: Genotyping was performed for *2, *3A and *3C.	Dose versus NM (corrected for 6-TGN concentrations): IM: 43%
ref. 20 - imm sup Ansari A et al. Influence of xanthi-	3	A total of 31 patients with Crohn's disease (26x NM, 4x IM, 1x PM), who used azathioprine ≥ 2 months. Co-medication: mesalazine (n=15).	

ne oxidase on thiopurine metabolism in Crohn's disease. Aliment Pharmacol Ther 2008;28:749-57. **ref. 20, continuation**	IM: AA PM: A(2)	IM versus NM: - decrease in median dose by 50% (from 2.0 to 1.0 mg/kg azathioprine per day) (NS) PM versus NM: - decrease in dose by 95.5% (from median 2.0 to 0.09 mg/kg azathioprine per day) (NS) N.B.: Genotyping was performed for *2, *3A and *3C.	Dose versus NM: IM: 50% PM: 4.5%
ref. 21 - imm sup Gardiner SJ et al. Thiopurine dose in intermediate and normal metabolizers of thiopurine methyltransferase may differ three-fold. Clin Gastroenterol Hepatol 2008;6:654-60.	3 IM: A	A total of 52 patients with Crohn's disease or ulcerative colitis (47x NM, 5x IM), were treated with azathioprine or mercaptopurine for 9 months. The average initial dose was 1 mg/kg azathioprine-equivalents per day. The dose was adjusted according to the 6-TGN concentration (target value 235-450 pmol/8x10^8 RBC) and the patient's clinical condition. Co-medication: mesalazine (78% of the patients). The median TPMT activity did not differ between the groups that did and did not use mesalazine. IM versus NM: - decrease in dose after 9 months by 50% (from 1.8 to 0.9 mg/kg azathioprine per day) (S) - increase in 6-TGN concentration after 9 months by 85% (from 273 to 505 pmol/8x10^8 RBC) (S). Increase after correction for dose and weight by 216% (from 183 to 578 pmol/8x10^8 RBC per mg/kg per day) (S). - no difference in clinical outcome - there was no difference in the percentage IM between the group of patients treated for 9 months and a group of 16 patients who had to withdraw from the study after a median of 1 month due to intolerance N.B.: Genotyping was performed for *2, *3A and *3C.	Dose versus NM (corrected for 6-TGN concentrations): IM: 32%
ref. 22 - imm sup Moloney FJ et al. The frequency and significance of thiopurine S-methyltransferase gene polymorphisms in azathioprine-treated renal transplant recipients. Br J Dermatol 2006;154:1199-200.	3 IM: AA	Of 407 kidney transplant patients (375x NM, 32x IM (28x *1/*3A, 1x *1/*3B, 3x *1/*3C)), 332 received azathioprine after transplantation (standard dose with initial dose of 2.5 mg/kg per day). 224 patients (217 NM, 24 IM) received AZA for > 5 years. As long-term AZA and UV light have a synergistic effect on the development of non-melanoma skin cancer, the relationship between TPMT activity and skin cancer was investigated. IM versus NM: - higher percentage of skin cancer with >5 years AZA (46% versus 41%, OR = 2.61 (NS)) - haematological toxicity necessitated withdrawal of AZA in 20% of the IM. The study does not state whether and how many NM had to withdraw from therapy.	Authors' conclusion: "This study suggests that possessing a variant TPMT gene may contribute to skin cancer risk in azathioprine-treated transplant patients but that such risk is overshadowed by other environmental and genetic factors known to predispose to skin cancer."
ref. 23 - imm sup Zelinkova Z et al. Inosine triphosphate pyrophosphatase and thiopurine s-methyltransferase genotypes relationship to azathioprine-induced myelosuppression.	4[#]	A total of 262 patients with Crohn's disease or ulcerative colitis (238x NM, 23x IM (17x *1/*3A, 6x *1/*3C), 1x PM (*3A/*3A)), received azathioprine (dose according to protocol; 50-250 mg/day (mean 132 mg/day) for 1-143 months (mean 35 months), co-medication mesalazine (55%), corticosteroids (79%), anti-TNF (13%)). Analysis was retrospective. - the frequency of mutant alleles was higher in the population with leukopenia than in the patients without leukopenia (20.8% versus 4% (S)).	Authors' conclusion: "ITPA 94C>A and TPMT polymorphisms are associated with AZA-related leukopenia in IBD patients. However, in terms of consequences

Clin Gastroenterol Hepatol 2006;4:44-9. **ref. 23, continuation**	(IM + PM): C PM: C(2)	- mutant alleles result in a higher risk of leukopenia < $3.0x10^9$/L: OR = 6.3 (S) - differences in AZA dose and co-medication between the group with leukopenia and the group without leukopenia were non-significant. - PM versus (IM + NM): more rapid development of leukopenia (within 2 weeks versus after an average of 7.1 months). Necessitated withdrawal from therapy. - the frequency of mutant alleles was not significantly higher in the population with hepatotoxicity than in the patients without hepatotoxicity (4.6% versus 9.1% (NS)).	for clinical practice, the only up-to-date known serious and preventable AZA-related adverse event is leukopenia resulting from low TPMT enzymatic activity in homozygous mutants."
ref. 24 - imm sup Jun JB et al. Thiopurine S-methyltransferase polymorphisms and the relationship between the mutant alleles and the adverse effects in systemic lupus erythematosus patients taking azathioprine. Clin Exp Rheumatol 2005;23:873-6.	3 IM: AA	94 SLE patients (86x NM, 8x IM (6x *1/*3C, 2x *1/*6)) received azathioprine 65.2 ± 22.1 mg/day for 94.9 ± 85.7 weeks. Analysis was retrospective. IM versus NM: - no difference in frequency of patients with adverse events (25.0% versus 24.4%) Genotyping for TPMT in 13 patients (8 RA, 5 SLE) with severe leukopenia after AZA: 12 were NM, 1 was IM (*1/*3C).	Authors' conclusion: "This study identified no statistical correlation between TPMT genotype and AZA toxicity."
ref. 25 - imm sup Stocco G et al. TPMT genotype and the use of thiopurines in paediatric inflammatory bowel disease. Dig Liver Dis 2005;37:940-5.	3 IM: A	A total of 70 children with Crohn's disease or ulcerative colitis (65x NM, 5x IM (4x *1/*3A, 1x *1/*2)), who used thiopurines ≥ 3 months, or who suffered adverse events caused by thiopurines. Medication: 52x azathioprine (1.0-4.0 mg/kg per day (median 2.0 mg/kg per day) over 0.5 – 85.0 months (median 19.6 months)), 18x 6-methylpurine (dose is converted for AZA, see there). Co-medication: 63x mesalazines. Analysis was retrospective. IM versus NM: - higher risk of intolerance to thiopurines (40% versus 26.2%, OR = 1.88 (NS)) - larger proportion of the tolerant patients exhibited a clinical response (3/3 versus 31/48) - decrease in dose required for clinical response (AZA or AZA-equivalent from median 2.0 to 1.6 mg/kg per day) (S by 20%).	Authors' conclusion: "There was no significant association between adverse effects of thiopurines and TPMT heterozygous genotype, but TPMT genotyping could be useful in establishing the most appropriate dose of thiopurines to start treatment. However, clinicians should still monitor patients being treated with these toxic medications, by careful surveil--lance of WBC or whole blood cell count and liver and pancreatic function, so as to detect the common forms of toxicity unrelated to TPMT genotype."
ref. 26 - imm sup Kurzawski M et al. The impact of thiopurine s-methyltransferase polymorphism on azathioprine-induced mye-	3#	112 kidney transplant patients (98x NM, 13x IM (10x *1/*3A, 2x *1/*2, 1x *1/*3C), 1x PM (*3A/*3C)) received azathioprine + cyclosporine + prednisone for 1 year. AZA dose was initially approx. 2.5 mg/kg per day and was reduced to 1.5 mg/kg per day during the first week. The AZA dose was adjusted if adverse events occurred. The cyclosporine dose was initially 7 mg/kg per day and was adjusted based on TDM.	Authors' conclusion: "Our results suggest that polymorphisms in TPMT gene may be responsible for

lotoxicity in renal transplant recipients. Ther Drug Monit 2005;27:435-41. **ref. 26, continuation**	IM: C PM: C(2)	Prednisone was administered according to standard immunosuppressant therapy. Patients received acetylsalicylic acid 75 mg/day during the 1st month. Co-medication varied between patients. Patients with allopurinol co-medication were excluded. IM versus NM: - increase in the frequency of episodes with leukopenia < $4.0x10^9$/L from 23.5% to 53.8% (S by 129%). - increase in the frequency of episodes with leukopenia < $3.0x10^9$/L from 11.3% to 38.5% (S by 241%). - decrease in average final dose of AZA from 1.5 to 1.11 mg/kg per day (NS) - no difference in episodes of acute transplant rejection PM: - developed 2x leukopenia < $3.0x10^9$/L after AZA 0.75 mg/kg per day. AZA was replaced by mycophenolic acid/tacrolimus.	approximately 12.5% of all leukopenia episodes in renal transplant recipients treated with azathioprine. Genotyping for the major TPMT variant alleles may be a valuable tool in preventing AZA toxicity and optimization of immunosuppressive therapy."
ref. 27 - imm sup Gardiner SJ et al. Two cases of thiopurine methyltransferase (TPMT) deficiency--a lucky save and a near miss with azathioprine. Br J Clin Pharmacol 2006;62:473-6.	2 PM: D PM: A	2 cases: - Patient 1 developed severe myelosuppression 8 weeks after the start of azathioprine 100 mg/day (approx. 1.4 mg/kg per day) for Crohn's disease. Recovery occurred after withdrawal of AZA and following infusions of RBCs, platelets and filgrastrim. Due to an error, the patient was again given azathioprine 100 mg/day 7 months later and again developed severe myelosuppression. He was found to be PM (*3/*3). - Patient 2 was found to be *3/*3 four days after starting azathioprine 50 mg/day (0.64 mg/kg per day) for ulcerative colitis. Azathioprine was stopped. After six months, treatment was started with azathioprine 12.5 mg 2x per week (equivalent to 0.05 mg/kg per day). This treatment resulted in clinical improvement within six months and 6-TGN concentrations of 250-400 pmol/$8x10^8$ RBC (within the target range of 235-450 pmol/$8x10^8$ RBC).	Maintenance dose versus a standard dose of AZA 2-2.5 mg/kg per day: PM: 2.2%
ref. 28 - imm sup Kurzawski et al. Severe azathioprine-induced myelotoxicity in a kidney transplant patient with thiopurine S-methyltransferase-deficient genotype (TPMT *3A/*3C). Transpl Int 2005;18:623-5.	2 PM: E	A kidney transplant patient developed myelosuppression two months after starting azathioprine (200 mg on day 1, 150 mg/day on day 2-10, followed by 50 mg/day) + cyclosporine (500 mg/day on day 1-8, followed by 350 mg/day) + prednisone (45 mg/day, gradual reduction to 20 mg/day after 2 weeks). Co-medication: acetylsalicylic acid 500 mg/day, verapamil, co-trimoxazole and cefuroxime. Recovery occurred after withdrawal of AZA. The patient again developed myelosuppression three weeks after starting AZA again (initially 75 mg/day, then reduced to 50 mg/day). AZA was replaced by mycophenolic acid. The patient was found to be a *3A/*3B.	Authors' conclusion: "Evaluation of TPMT polymorphism in patients treated with thiopurine drugs should be mandatory in order to optimize therapy."
ref. 29 - imm sup Fabre MA et al. The impact of thiopurine S-methyltransferase polymorphisms on azathioprine dose 1 year after renal transplantation. Transpl Int 2004;17:531-9.	4 IM: B	172 kidney transplant patients (160x NM, 12x IM (11x *1/*3A, 1x *1/*3C)), received azathioprine (initial dose 1.5 mg/kg per day) in combination with cyclosporine and prednisolone for 1 year. Co-medication: acetylsalicylic acid 75 mg/day during the 1st month, co-trimoxazole. Patients with allopurinol co-medication were excluded. - No serious adverse events, such as bone marrow aplasia or hepatotoxicity occurred. IM versus NM: - increase in the percentage of patients requiring dose reduction due to leukopenia < $4.0x10^9$/L from 30% to 58% (S by 93%)	Authors' conclusion: "We concluded that when azathioprine is administered at an initial dose of 1.5 mg/kg per day, both coding and promoter TPMT polymorphisms influence the dose tolerated."

ref. 29, continuation	high NM: A	- decrease in the average dose after 1 year versus the initial dose from 82.6% to 67.9% (NS) - number of patients with ≥ 1 acute rejection episode is comparable: 50% versus 43% (NS) - NM with ≤ 10 "variable number tandem repeats" in their TPMT promoters versus NM with ≥ 11 repeats (n=22): - decrease in the percentage of patients requiring dose reduction from 59% to 25% (S by 58%) - increase in the average dose after 1 year versus the initial dose from 68.9% to 84.6% (S by 23%) - number of patients with ≥ 1 acute rejection episode is comparable: 41% versus 50% (NS) N.B.: The relationship between promoter polymorphisms and TPMT activity is controversial. Previous research has demonstrated an inverse relationship between *in vitro* TPMT activity and total number of repeats.	
ref. 30 - imm sup Gearry RB et al. Thiopurine S-methyltransferase (TPMT) genotype does not predict adverse drug reactions to thiopurine drugs in patients with inflammatory bowel disease. Aliment Pharmacol Ther 2003;18:395-400.	3[#] IM: AA PM: E(2)	50 patients with inflammatory bowel disease, who had to stop azathioprine or 6-mercaptopurine due to adverse events, were compared to 50 patients who tolerated azathioprine/6-mercaptopurine (dose unknown). - The 50 intolerant patients were found to be 44x NM, 5x IM (5x *1/*3) and 1x PM (*3/*3). The 50 tolerant patients were found to be 47x NM and 3x IM (*1/*3). There was a trend towards more adverse events for IM + PM (NS). - Of the two patients with myelosuppression, one was IM and the other PM. The PM had severe pancytopenia, necessitating hospital admission. Hospital admission was also necessary for the IM. - The patients with the most common adverse event (hepatitis, 30%) were all NM. NB: *3 is *3A or *3C.	Authors' conclusion: "There was a slight trend for more frequent TPMT mutations in the patients with adverse reactions, but this was not statistically significant. Most patients with reactions did not have gene mutations."
ref. 31 - imm sup Gilissen LP et al. Some cases demonstrating the clinical usefulness of therapeutic drug monitoring in thiopurine-treated inflammatory bowel disease patients. Eur J Gastroenterol Hepatol 2004;16:705-10.	2 IM: A PM: D	This article describes 5 cases, of which 2 with TPMT polymorphisms (1x IM (*1/*3A), 1x PM (*3A/*3A)). - Patient with *1/*3A genotype (60 years, ulcerative colitis) received 6-mercaptopurine 50 mg/day (0.7 mg/kg per day) + olsalazine 1000 mg 3x per day. After two months, it was decided to reduce the 6-MP to 25 mg per day, because the 6-TGN concentrations (628 pmol/8x10^8 RBC) were high compared to the 6-MMP concentrations (362 pmol/8x10^8 RBC). At this dose, the 6-TGN concentration was 417 pmol/8x10^8 RBC, whilst 6-MMP was not detectable. Disease activity was in remission. - Patient with *3A/*3A genotype (32 years, ulcerative colitis) received 6-mercaptopurine 50 mg/day (0.5 mg/kg per day). Therapy was stopped due to suspected PM phenotype, because 6-TGN concentrations were extremely high (1284 pmol/8x10^8 RBC). The patient was then treated in a different hospital with azathioprine 50 mg/day and developed severe leukopenia after several weeks. Treatment with 6-thioguanine is being considered.	Authors' conclusion: "Heterozygous patients like case 3 should have a dose reduction and intensive TDM, while homozygous poor metabolizers (TPMTL=L) like case 4 are candidates for treatment with 6-TG or a significant dose reduction, according to a recent report." Maintenance versus initial dose: IM: 50%
ref. 32 - imm sup Kaskas BA et al. Safe treatment of thiopurine S-methyl-	2 PM: F	3 cases with Crohn's disease: - Patient 1 developed severe myelosuppression 8 weeks after the start of azathioprine 1.3 mg/kg per day. Recovery occurred after withdrawal of AZA. 4 years later, AZA 0.29 mg/kg	Authors' conclusion: "We illustrate this with three cases

transferase deficient Crohn's disease patients with azathioprine. Gut 2003;52:140-2. **ref. 32, continuation**		per day for 7 months had a good therapeutic effect. He was found to be PM (*3A/*3A). - Patient 2 developed tonsillitis with moderate leukopenia 1.5 years after starting azathioprine 1 mg/kg per day. Recovery occurred after withdrawal of AZA. Five months later she received AZA 0.25 mg/kg per day + methylprednisolone (dose unknown). Due to very high 6-TGN concentrations (1014 pmol/8x10^8 RBC), the AZA was reduced to 0.20 mg/kg per day. Following a single infusion with infliximab, the patient was in continuous remission for over a year, without adverse events, on AZA 0.16 mg/kg per day + budesonide 9 mg/day. She was found to be PM (*3A/*3A). - Patient 3 was asymptomatic for 7 years after starting azathioprine 0.71 mg/kg per day. After genotyping (PM: *3A/*3C), the AZA was reduced to 0.26 mg/kg per day. 6-TGN concentrations were 797-884 pmol/8x10^8 RBC after dose reduction.	where treatment has been successful and toxicity has been avoided by carefully titrating the drug dose. Thus very low TPMT activity demands pharmacogenetically guided dosing." Maintenance versus initial dose: PM: 16-37% Maintenance dose versus a standard dose of AZA 2-2.5 mg/kg per day: PM: 11%
ref. 33 - imm sup Ansari A et al. Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. Aliment Pharmacol Ther 2002;16:1743-50.	3 IM: C high NM: C	A total of 106 patients with Crohn's disease or ulcerative colitis, who were using or had used azathioprine, were selected retrospectively. 96x NM (30x *1/*1, the rest only phenotypic determination) and 10x NM (8x *1/*3A; 2x *1/*3C). Medication: azathioprine 50-175 mg/day (median: 100 mg/day; mean 1.69 mg/kg per day) over 1-108 months (median: 6 months). IM versus NM: - is more often intolerant to azathioprine: 50% versus 16%, OR = 5.4 (S) - in both cases, one person with myelosuppression High NM (> 14 U/mL RBC) versus low NM (10-13.9 U/ml RBC): - lower chance of complete therapeutic response: OR = 0.21 (S)	Authors' conclusion: "Inflammatory bowel disease patients with intermediate TPMT activity have an increased risk of azathioprine toxicity. Conversely, very high TPMT activity predicts treatment failure."
ref. 34 - imm sup Langley PG et al. Thiopurine methyltransferase phenotype and genotype in relation to azathioprine therapy in autoimmune hepatitis. J Hepatol 2002;37:441-7.	3 IM: A	A total of 72 patients with auto-immune hepatitis (94x NM, 15x IM, 1x PM) received azathioprine 1 mg/kg per day + prednisolone 0.5 mg/kg per day. The prednisolone was reduced to the lowest dose required to achieve biochemical remission. For patients with biochemical and clinical remission > 1 year on maintenance dose, the AZA was increased to 2 mg/kg per day and the steroids were tapered. - TPMT activity was lower in intolerant patients (median 14.0 U/mL; n=15) than in patients on AZA 2 mg/kg per day (median 19.8 U/mL; n=28) (S, decrease by 29%) - TPMT activity was lower in patients who remain in remission with only AZA 2 mg/kg per day (median 19.8 U/mL; n=28) than in patients who also require corticosteroids (median 21.6 U/mL; n=29) (S, decrease by 8.3%) N.B.: TPMT activity was determined by phenotyping and checked by genotyping in 53/72 patients (for *3A, *3B and *3C, not for *2). There were seven patients with *3A and three with *3B. The phenotypes of these patients were 6x IM, 3x NM and 1x PM. 3/46 patients who were genotypically NM, were phenotypically IM. The proportion of genotypically IM patients was smaller with increasing TPMT activity in the group (5/15; 3/28 and 2/29 respectively).	Authors' conclusion: "TPMT phenotyping or genotyping may be advisable before institution of azathioprine therapy in AIH but neither approach invariably predicts response to the drug."

ref. 35 - imm sup Regueiro M et al. Determination of thiopurine methyltransferase genotype or phenotype optimizes initial dosing of azathioprine for the treatment of Crohn's disease. J Clin Gastroenterol 2002;35:240-4.	3 IM: A	For a total of 59 patients with Crohn's disease (52x NM, 7x IM), initial dose of azathioprine was based on the TPMT genotype. The 45 NM initially received AZA 2-2.5 mg/kg per day (mean 2.35 mg/kg per day). The 7 NM received < 2.0 mg/kg per day (mean 1.28 mg/kg per day). The 7 IM started at AZA 1-1.5 mg/kg per day. Co-medication: 42x mesalazine. Patient data from the first three months of therapy were analysed retrospectively. - None of the patients developed acute leukopenia. - thirteen patients (22%) developed adverse events that necessitated withdrawal of therapy or dose reduction: One IM with AZA 1.5 mg/kg per day (1/7 IMs = 14%) 10 NM with AZA > 2 mg/kg per day (10/45 IMs = 22%) 2 NM with AZA < 2 mg/kg per day (2/7 = 28%) - The average number of leukocytes decreased for: NM with AZA 2-2.5 mg/kg per day (S) IM with AZA 1-1.5 mg/kg per day (S). No significant decrease was found for NM with AZA < 2 mg/kg per day. - There was no significant difference in the number of leukocytes between individuals who were using AZA in combination with mesalazine and individuals who were using AZA alone. The distribution of TPMT activity and AZA doses was comparable in the groups with and without co-medication. N.B.: TPMT activity was partially determined by phenotyping (42%) of the patients and partially by genotyping.	Authors' conclusion: "Patients with Crohn's disease and normal TPMT activity who were started on high-dose AZA (2-2.5 mg/kg/d) and patients with intermediate enzyme activity who were started on reduced doses of AZA did not develop acute leukopenia."
ref. 36 - imm sup Pandya B et al. Azathioprine toxicity and thiopurine methyltransferase genotype in renal transplant patients. Transplant Proc 2002;34:1642-5.	4 IM: C	88 kidney transplant patients (76x NM, 12x IM (6x *1/*3A, 3x *1/*3B, 3x *1/*3C)), were treated with azathioprine (initial dose 2.0 mg/kg per day). Patients with allopurinol or antithymocyte globulin as co-medication and patients with active cytomegalovirus infection or other diseases were excluded from the analysis. IM versus NM: - increase in the percentage of patients that developed leukopenia < $3.5x10^9$/L from 16% to 58.3% (S by 264%) - decrease in the average leukocyte concentration from $7.2x10^9$/L to $4.0x10^9$/L (S by 44%) - larger proportion of patients stopped with AZA within three months due to leukopenia < $3.5x10^9$/L (S) *1/*3A versus *1/*3B versus *1/*3C: - 5/6 (83%) versus 2/3 (67%) versus 0/3 (0%) of the patients developed leukopenia (significance not reported)	Authors' conclusion: "This shows that TPMT genotyping can be a quick and easy way to screen patients before initiating azathioprine therapy in renal transplant recipients and may be a valuable aid in clinical decision making to reduce the risk of haematologic side effects."
ref. 37 - imm sup Campbell S et al. Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low-dose azathioprine. Aliment Pharmacol Ther 2002;16:389-98.	3 IM: A	TPMT activity was determined in 87 patients with inflammatory bowel disease, of which 63 were using azathioprine and 24 had stopped using azathioprine due to adverse events. Co-medication: included mesalazine. - The average TPMT activity was lower in patients who had stopped treatment due to neutropenia than in patients who developed other adverse events (S) - The average TPMT activity was not lower in the AZA-intolerant patients than in the patients using AZA. - In a group of 34 patients who used low-dose AZA for more than one year, the average TPMT activity was lower for the patients who did not exhibit any exacerbations versus the	Authors' conclusion: "The mean thiopurine methyltransferase activity was significantly lower in patients on a low dose of azathioprine in remission compared with those who relapsed. The thiopurine methyltransferase activi-

ref. 37, continuation		patients with exacerbations: 19.8 versus 27.6 nmol/mL RBC per hour (S, decrease by 28%). The AZA dose (median 1.5 mg/kg per day) and the median duration of the treatment was comparable in both groups. - In this group, the time to first exacerbation was longer for IM than for NM (S). - For the group of 63 patients who, on average, used a higher AZA dose (median 1.75 mg/kg per day), the same trend was observed in the relationship between time to first exacerbation and TPMT activity (NS).	ty was significantly lower in patients who discontinued azathioprine due to neutropenia than in those who discontinued due to other side effects."
ref. 38 - imm sup Colombel JF et al. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. Gastroenterology 2000;118:1025-30.	3 IM+PM: AA	A total of 41 patients with Crohn's disease, who developed leukopenia <3.0x10^9/L or thrombocytopenia <100 x10^9/L during treatment with azathioprine 50-200 mg/day (median 125 mg/day) or 6-mercaptopurine 50-150 mg/day (median 62.5 mg/day). Following myelosuppression, the treatment was stopped in 83% of the patients and the dose was reduced by ≥ 50% in 17% of the patients. Co-medication varied. - four patients (10%) were found to be PM (1x *2/*3A, 1x *3A/*3A, 1x *3A/*3C, 1x *3C/*3C) seven patients (17%) were found to be IM (3x *1/*3A, 2x *1S/*3A, 1x *1/*2, 1x *1/*3C) 29 patients (71%) were found to be NM and one patient had a previously unknown allele (*1/*10). Total: 27% IM + PM versus 10% in a European control population (significance unknown). - severe leukopenia (<2.0x10^9/L) occurred in 3/4 PM patients (75%) 2/7 IM patients (29%) 12/29 NM patients (41%) (significance unknown) - there was no clear correlation between AZA/6-MP dose and the severity of leukopenia PM versus NM: - bone marrow toxicity after median 1 month versus median 3 months. IM versus NM: - bone marrow toxicity after median 4 months versus median 3 months.	Authors' conclusion: "Twenty-seven percent of patients with CD and myelosuppression during azathioprine therapy had mutant alleles of the TPMT gene associated with enzyme deficiency. Myelosuppression is more often caused by other factors. Continued monitoring of blood cell counts remains mandatory in patients treated with azathioprine."
ref. 39 - imm sup Black AJ et al. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. Ann Intern Med 1998;129:716-8.	3 IM: C	A total of 66 patients (61x NM, 5x IM (5x *1/*3A)) with rheumatic conditions were treated with azathioprine 2-3 mg/kg per day (sometimes in combination with corticosteroids). IM versus NM: - decrease in median therapy duration from 39 to 2 weeks (S by 95%) - increased frequency of leukopenia <3.5x10^9/L as the cause of therapy withdrawal from 0% to 100%. No haematological abnormalities were observed in NM. The reasons for therapy withdrawal included other adverse events (nausea, hepatotoxicity) (33% of the patients) and lack of efficacy (30%).	Authors' conclusion: "Analysis of thiopurine methyltransferase genotype is a quick way to identify patients at risk for acute toxicity from azathioprine."
ref. 40 - imm sup/ cytostat Higgs JE et al. Are patients with intermediate TPMT activity at increased risk of myelosup-	3	Systematic review of 67 studies and meta-analysis of the data from 47 studies with patients who used azathioprine or mercaptopurine for various conditions. The total number of patients in the meta-analysis was 4,306, of which 434 were IM (determined by phenotyping or genotyping). Of the studies in the meta-analysis, ten were included as a reference in this risk analysis (Black 1998, McLeod 1999, Ansari 2002, Lang-	Authors' conclusion: "This meta-analysis suggests that individuals with both intermediate and absent TPMT

pression when taking thiopurine medications? Pharmacogenomics 2010;11:177-88. **ref. 40, continuation**	PM: E IM: C	ley 2002, Pandya 2002, Gearry 2003, Fabre 2004, Jun 2005, Stocco 2005 and Zelinkova 2006). *Systematic review* PM (phenotypic or genotypic): - Out of the total 43 PM, 86% developed severe myelosuppression. This was 7% for all patients in this and another meta-analysis. Dose adjustment: Two randomised controlled trials based the dose of azathioprine on the TPMT activity. However, one had no control group without dose adjustment and the other had no IM, so as a result it is not clear whether dose reduction for IM reduces the risk of myelosuppression. *Meta-analysis* IM versus NM (phenotypic or genotypic): - higher risk of leukopenia: OR = 4.19 (CI: 3.20-5.48) Number needed to test: From the OR and the 7% incidence of myelosuppression in the control group, it was calculated that six patients would have to be tested to detect one patient with an increased risk of leukopenia. The authors indicate that the studies looked at mild leukopenia instead of severe leukopenia, neutropenia or infection. Mild leukopenia can also be a sign of effective treatment, instead of a clinically relevant adverse event.	activity have an increased risk of developing thiopurine-induced myelosuppression, compared with individuals with normal activity." "This study highlights that the increased risk of myelosuppression for intermediate-activity patients, while present, is low and should not preclude the use of thiopurine medications."
ref. 41 - cytostat Stanulla M et al. Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. JAMA 2005;293:1485-9.	4 IM: A PM: A	A total of 810 ALL patients (755x NM, 55x IM (42x *1/*3A, 9x *1/*3C, 2x *1/*2, 1x *1/*9) were treated with 6-mercaptopurine 60 mg/m^2 per day + cyclophosphamide i.v. + cytarabine i.v. + methotrexate intrathecal for four weeks. Remaining leukaemia cells were measured before and after this consolidation treatment. IM versus NM: - lower frequency of remaining leukaemia cells above the detection limit (1 leukaemia cell per 10,000 cells): 9.1% versus 22.8%, RR = 0.34 (S) - no difference in haematological toxicity and hepatotoxicity. Four PM (2x *3A/*3A, 1x *2/*3A, 1x *3A/*11) received a 10x reduced dose of 6-MP. The frequency of patients with remaining leukaemia cells above the detection limit was 25%.	Authors' conclusion: "TPMT genotype has a substantial impact on minimal residual disease after administration of mercaptopurine in the early course of childhood ALL, most likely through modulation of mercaptopurine dose intensity." Dose versus NM: IM: 100% PM: 10%
ref. 42 - cytostat Schaeffeler et al. A novel TPMT missense mutation associated with TPMT deficiency in a 5-year-old boy with ALL. Leukemia 2003;17:1422-4.	2 PM: A	A boy with ALL was found to be PM after phenotyping. Genotyping initially only revealed one mutant allele (*3A). Sequencing revealed a new mutant allele (*11). He was treated with 6-mercaptopurine at 15% of the standard dose. He exhibited no 6-MP-related toxicity.	Authors' conclusion: "Large-scale genotype-phenotype correlation studies are needed to evaluate the predictive power of TPMT genotyping before a sole genotype-guided approach of thiopurine medication will become a clinical reality." Dose versus the standard: PM: 15%

ref. 43 - cytostat Evans WE et al. Preponderance of thiopurine S-methyltransferase deficiency and heterozygousity among patients intolerant to mercaptopurine or azathioprine. J Clin Oncol 2001;19:2293-301.	3 IM+PM: E	A total of 23 children with excessive toxicity to thiopurines: 2x auto-immune diseases treated with azathioprine; 19x ALL treated with mercaptopurine; 1x ALL with thioguanine; 1x ALL with 6-MP and 6-TG during various periods. - The patients were found to be 6x PM, 9x IM and 8x NM. The frequency of 65.2% IM + PM within these toxic patients is higher than the expected frequency of 10% within the general population (S). - Toxicity. Haematological toxicity alone or in combination with other toxicities occurred in 21/23 (90%) of the patients. No significant differences between the three TPMT phenotypes were found for: - the number of weeks of therapy before toxicity occurred - the occurrence of various types of toxicity (haematopoietic toxicity, hepatotoxicity or other toxicity) - the period required for recovery from neutropenia and resumption of the treatment - the treatments required for recovery (blood transfusion, thrombocyte transfusion, hospital admission, antibiotics, G-CSF). - Following dose reduction of 6-MP or 6-TG, the patients tolerated the therapy without acute toxicity and 50-62% could be treated with a complete dose of their other chemotherapy. Median dose reduction: - NM: 8.3% (from median 350 to median 280 mg/m^2 per week) - IM: 66.7% (from median 525 to median 175 mg/m^2 per week) - PM: 90.8% (from median 350 to median 32 mg/m^2 per week) The initial doses, the reduced doses and the percentage reduction all varied between the three TPMT phenotypes (S). - 2/17 patients in remission experienced an exacerbation: 1x NM and 1x IM. N.B.: TPMT activity was determined by phenotyping and confirmed by genotyping for 6/6 PM (6x *3A/*3A), 3/9 IM (2x *1/*3A, 1x *1/*3C) and 6/8 NM. No TPMT mutation (*2, *3A, *3B or *3C) was found for 5/9 IM. One IM was not tested.	Authors' conclusion: "There is a significant (> six-fold) overrepresentation of TPMT deficiency or heterozygosity among patients developing dose-limiting hematopoietic toxicity from therapy containing thiopurines. However, with appropriate dose adjustments, TPMT-deficient and heterozygous patients can be treated with thiopurines, without acute dose-limiting toxicity." Median maintenance dose versus NM: IM: 63% PM: 11%
ref. 44 - cytostat Relling MV et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 1999;91:2001-8.	4# PM: E(2) IM: E	A total of 180 children with ALL (161x NM, 17x IM, 2x PM) received 6-mercaptopurine 75 mg/m^2 per day + methotrexate 40 mg/m^2 per week i.v. or i.m. for 2.5 years. During the first year, the therapy is interrupted every six weeks for treatment with either high-dose methotrexate or teniposide + cytarabine. Dose reduction in the event of myelosuppression. PM: reduction in dose of 6-MP from 75 mg/m^2 per day to 10 mg/m^2 3x per week. IM: reduction to dose resulting in leukocytes < 4x10^9/L and neutrophils > 0.3x10^9/L. - 6-TGN concentrations were inversely proportional to TPMT activity (S): NM: 417 ± 179 pmol/8x10^8 RBC IM: 963 ± 752 pmol/8x10^8 RBC PM: 3565 ± 1282 pmol/8x10^8 RBC - PM tolerated a complete dose of 6-MP only 7% of the time, IM 65% and NM 84%. - IM had a greater risk of missing therapy weeks with 6-MP	Authors' conclusion: "Lowering doses of 6-mercaptopurine in TPMT heterozygotes and in deficient patients allowed administration of full protocol doses of other chemotherapy while maintaining high thioguanine nucleotide concentrations. We conclude that genetic polymorphism in TPMT is an important determinant of

ref. 44, continuation		than NM (S). - The percentage of patients requiring dose reduction of 6-MP was 100% for PM, 35% for IM and 7% for NM (S). - The final doses of 6-MP were: NM: 528 ± 90 mg/m^2 per week IM: 449 ± 160 mg/m^2 per week PM: 72 ± 60 mg/m^2 per week N.B.: TPMT activity was determined by phenotyping and confirmed by genotyping for 18 NM, 8 IM (8x *1/*3A) and 2 PM (1x *2/*2 and 1x *2/*3A).	mercaptopurine toxicity, even among patients who are heterozygous for this trait." Maintenance dose versus NM: PM: 14%
ref. 45 - cytostat McLeod HL et al. Analysis of thiopurine methyltransferase variant alleles in childhood acute lymphoblastic leukaemia. Br J Haematol 1999;105:696-700.	3# IM: AA PM: E(2)	A total of 147 children with ALL (130x NM, 16x IM (14x *1/*3A, 2x *1/*3C), 1x PM (*3A/*3A)) received 6-mercaptopurine (complete dose 75 mg/m^2 per day, dose was reduced according to protocol in the event of toxicity). Sufficient data for analysis were obtained from 94 children (83x NM, 10x IM, 1x PM). IM versus NM: - no significant difference in the percentage of the maintenance period in which no treatment could be given due to haematological toxicity (median 9.5% versus 11%, NS) - no statistical difference in the percentage of the time that the complete dose could be given or that a reduced dose was given (NS). PM versus NM: - therapy could more frequently not be given due to toxicity (53% versus 11% of the time). - patient is permanently bold due to therapy.	Authors' conclsion: "Prospective identification of TPMT genotype may be a promising tool for decreasing excessive haematological toxicity in individuals with low activity."
ref. 46 - cytostat Andersen JB et al. Pharmacokinetics, dose adjustments, and 6-mercaptopurine/methotrexate drug interactions in two patients with thiopurine methyltransferase deficiency. Acta Paediatr 1998;87:108-11.	2 PM: F	- Patient (6.5 years) developed four episodes of severe pancytopenia and bone marrow hypoplasia two weeks after starting/resuming 6-mercaptopurine 60 mg/m^2 per day. Blood counts recovered after two weeks without treatment. Due to suspected TPMT deficiency, 25% of the standard dose was subsequently used for the intermittent 6-MP treatment during consolidation. TPMT deficiency was confirmed (genotype *3A/*3A and very low TPMT activity). Within 2-3 weeks after starting the maintenance therapy (6-mercaptopurine 7.5 mg/m^2 per day + methotrexate 20 mg/m^2 per week), the patient again developed severe pancytopenia. The 6-MP was titrated to ensure that platelets were approx. 100x10^9/L and leukocytes 1.5-3.5x10^9/L. The average dose was then 3.3 mg/m^2 per day. The patient is still in the first ALL remission two years and two months after diagnosis. - Patient (4 years) developed three episodes of severe pancytopenia and severe bone marrow hypoplasia 4-5 weeks after starting/resuming 6-mercaptopurine 75 or 37.5 mg/m^2 per day + methotrexate 20 mg/m^2 per week. Recovery occurred within 2-5 weeks of stopping the treatment. Due to suspected TPMT deficiency, 6-mercaptopurine was resumed at 12 mg/m^2 per day + methotrexate 20 mg/m^2 per week. The 6-MP was titrated to ensure that platelets were approx. 100x10^9/L and leukocytes 1.5-4.0x10^9/L. The dose was eventually maintained at 15-25 mg/m^2 per day (average 20.0 mg/m^2 per day). TPMT deficiency was confirmed (genotype *3A/*3C and very low TPMT activity).	Authors' conclusion: "On the basis of the present findings and the previously reported data on TPMT-deficient patients we would suggest the following guidelines: (i) patients proven or suspected to be TPMT-deficient should be started on a dose of 6MP that is 1/10th of the protocol recommendations; (ii) the dose should then be adjusted on the basis of the occurrence of myelotoxicity; and (iii) for patients experiencing myelotoxicity following HDMTX, a further reduction of 6MP 2 weeks prior to HDMTX in order to reduce

ref. 46, continuation		The patient is still in the first ALL remission six years after diagnosis. - For both patients on a reduced dose of 6-MP, the RBC 6-TGN concentration is several times higher than at 100% dose for normal patients. - In both patients, blood counts drop rapidly after administration of methotrexate (1 or 5 g/m^2 i.v. in 24 hours). For the first patient, this resulted in a further three episodes of pancytopenia with reduced dose of 6-MP, for which treatment was stopped temporarily. His methotrexate was reduced to 15 mg/m^2 per week. No adjustment of the treatment was required for the second patient. He did not experience this decrease without 6-MP. The authors postulate that inhibitors of *de novo* purine synthesis could increase the toxic effects of 6-TGN.	intracellular 6TGN may ameliorate bone-marrow suppression (unpublished data)." Dose versus the standard dose: *3A/*3A: 4.4% *3A/*3C: 26.7%
ref. 47 - imm sup SmPC Imuran (azathioprine) 04-12-18.	0 PM: E	<u>Dose</u>: Patients with a congenital low or absent activity of thiopurine S-methyltransferase (TPMT) are at increased risk of severe azathioprine toxicity with conventional doses of azathioprine. These patients usually require substantial dose reduction. The optimum initial dose for patients with homozygous TPMT deficiency has not been determined. Most patients with heterozygous TPMT deficiency are able to tolerate the recommended doses of azathioprine, but dose reduction may be required for some. Tests are available for genotyping and phenotyping for TPMT. <u>Warning</u>: In rare cases, individuals have a congenital deficiency of the enzyme thiopurine S-methyltransferase (TPMT). They can be unusually sensitive to the myelosuppressive effect of azathioprine and prone to developing rapid myelosuppression following initiation of azathioprine treatment. This problem can be exacerbated by simultaneous administration of medicines that inhibit TPMT, such as: olsalazine, mesalazine or sulphasalazine. A possible link has also been reported between reduced TPMT activity and secondary leukaemia and myelodysplastic syndrome in individuals who received 6-mercaptopurine (the active metabolite of azathioprine) in combination with other cytotoxic drugs. There are laboratories that offer tests for TPMT deficiency, but it has not been demonstrated that these tests can detect all patients at risk of severe toxicity. Therefore, it remains essential to monitor blood counts closely. <u>Pharmacology:</u> Patients with variants both the NUDT15 and the TPMT enzyme tolerate thiopurines significantly less than patients with risk alleles of only one of these two genes. <u>Pharmacokinetics</u>: The activity of TPMT is inversely proportional to the concentration of thioguanine nucleotides from 6-mercaptopurine in red blood cells, with higher concentrations of thioguanine nucleotides resulting in greater reductions in the numbers of white blood cells and neutrophils. People with TPMT deficiency develop very high, cytotoxic concentrations of thioguanine nucleotides. The allele pattern of a patient can be determined by genotype testing. According to the current knowledge, three alleles – TPMT*2, TPMT*3A and TPMT *3C – are responsible for approximately 95% of individuals with reduced TPMT activity. Approximately 0.3% of the patients (1:300) have two non-functional alleles of the TPMT gene (homozygous deficient) and have little or no detectable enzyme activity. Approxima-	

ref. 47, continuation		tely 10% of the patients have one non-functional TPMT allele (heterozygous), which results in low or intermediate TPMT activity, and 90% of the patients have normal TPMT activity and two functional alleles. There could also be a group, approximately 2%, with very high TPMT activity. By testing the phenotype, the concentration of thiopurine nucleotides or the TPMT activity in red blood cells can be determined; this can also have informative value. Adverse events: Use of azathioprine can be accompanied with a dose-dependent, generally reversible, reduction of bone marrow function. In most cases, this manifests as leukopenia, sometimes however also as anemia and thrombocytopenia, and rarely as agranulocytosis, pancytopenia and aplastic anemia. This occurs most often in patients with a predisposition for myelotoxicity, such as patients with thiopurine S-methyltransferase (TPMT) deficiency.	
ref. 48 - cytostat SmPC Puri-Nethol (mercaptopurine) 13-02-19.	0 PM: E	Dose: Patients with a congenital low or absent thiopurine S-methyltransferase (TPMT) activity are at increased risk of severe toxicity with conventional doses of 6-mercaptopurine, and usually require a substantial dose reduction. The optimum initial dose for homozygous deficient patients has not been determined. Warning: Rare cases of individuals with a congenital deficiency of the enzyme thiopurine S-methyltransferase (TPMT) are known. These patients can be exceptionally sensitive to myelosuppression by 6-mercaptopurine and can therefore develop myelosuppression very soon after the start of treatment with Puri-Nethol. This problem can be exacerbated by simultaneous administration of medicines that inhibit TPMT, such as olsalazine, mesalazine or sulphasalazine. A possible link has also been reported between reduced TPMT activity and secondary leukaemia and myelodysplastic syndrome in individuals who received 6-mercaptopurine in combination with other cytotoxic drugs. Some laboratories offer tests to detect TPMT deficiency. However, these tests have not demonstrated that they are able to identify all patients at risk of severe toxicity. Therefore, monitoring of the blood counts is still essential. Pharmacology: Patients with variants both the NUDT15 and the TPMT enzyme tolerate thiopurines significantly less than patients with risk alleles of only one of these two genes. Pharmacokinetics: Several polymorphisms of thiopurine S-methyltransferase (TPMT) can affect metabolic routes of 6-mercaptopurine. Patients with very low or absent TPMT activity (approximately 0.3% of patients) can develop very high cytotoxic thioguanine nucleotide concentrations.	
ref. 49 - imm sup SmPC Imuran (azathioprine), USA, 20-12-18.	0	Dose: *Patients with TPMT and/or NUDT15 deficiency* 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 complete blood count 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	

ref. 49, continuation	IM: E PM: F	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. Death associated with pancytopenia has been reported in patients with absent TPMT activity receiving azathioprine. In patients with severe myelosuppression, consider evaluation for TPMT and NUDT15 deficiency. Consider alternative therapy in patients with homozygous TPMT or NUDT15 deficiency and reduced dosages in patients with heterozygous deficiency. Precautions: TPMT and NUDT15 Testing: 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. Accurate phenotyping (red blood cell TPMT activity) results are not possible in patients who have received recent blood transfusions. 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 concentrations 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. Approximately 0.3% (1:300) of patients of European or African ancestry have two loss-of-function alleles of the TPMT gene and have little or no TPMT activity (homozygous deficient or poor metabolizers), and approximately 10% of patients have one loss-of-function TPMT allele leading to intermediate TPMT activity (heterozygous deficient or intermediate metabolizers). The TPMT*2, TPMT*3A, and TPMT *3C alleles account for about 95% of individuals with reduced levels of TPMT activity. Adverse reactions: Patients with low or absent TPMT or NUDT15 activity are at increased risk for severe, life-threatening myelosuppression from Imuran.	
ref. 50 - cytostat SmPC Purixan (mercaptopurine), USA, 20-02-18.	0	Dose: 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 standard Purixan dosage. Reduce initial dosage in patients who are known to have homozygous TPMT or NUDT15 deficiency. *Heterozygous deficiency in TPMT and/or NUDT15* Reduce the PURIXAN dosage based on tolerability. Most	

ref. 50, continuation	IM: E PM: E	patients with heterozygous TPMT or NUDT15 deficiency tolerate recommended mercaptopurine doses, but some require dose reduction based on toxicities. Patients who are heterozygous for both TPMT and NUDT15 may require more substantial dosage reductions. Warning: Evaluate patients with repeated severe myelosuppression for thiopurine S-methyltransferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency. TPMT genotyping or phenotyping (red blood cell TPMT activity) and NUDT15 genotyping can identify patients who have reduced activity of these enzymes. Patients with homozygous TPMT or NUDT15 deficiency require substantial dosage reductions of Purixan. 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 range for patients with TPMT and/or NUDT15 deficiency on mercaptopurine maintenance therapy (as a percentage of the planned dosage) was as follows: heterozygous for either TPMT or NUDT15, 50-90%; heterozygous for both TPMT and NUDT15, 30-50%; homozygous for either TPMT or NUDT15, 5-10%. Approximately 0.3% (1:300) of patients of European or African ancestry have two loss-of-function alleles of the TPMT gene and have little or no TMPT activity (homozygous deficient or poor metabolizers), and approximately 10% of patients have one loss-of-function TPMT allele leading to intermediate TPMT activity (heterozygous deficient or intermediate metabolizers). The TPMT*2, TPMT*3A, and TPMT *3C alleles account for about 95% of individuals with reduced levels of TPMT activity. Consider all clinical information when interpreting results from phenotypic testing used to determine the level of thiopurine nucleotides or TPMT activity in erythrocytes, since some coadministered drugs can influence measurement of TPMT activity in blood, and blood from recent transfusions will misrepresent a patient's actual TPMT activity.	Dose versus the standard dose: IM: 50-90% PM: 5-10%

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

Risk group	Use of TPMT inhibitors (aminosalicylates: mesalazine, olsalazine or sulphasalazine) or xanthine oxidase inhibitors (allopurinol, febuxostat), use of inhibitors of *de novo* purine synthesis (methotrexate), NUDT15 IM or PM (frequent in East Asian patients) Note: results regarding the effect of the aminosalicylates 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: effi-

	cacy and safety results of a randomised, double-blind, double-dummy, multicentre trial. Gut 2010;59:752-9).

Comments:

- Due to the large number of articles about TPMT and AZA/6-MP, a selection was made for the status report. The selection for 2010 took place according to the following criteria:
  - clinical effects
  - genotyping
  - either studies involving more than 10 IM or more than 2 PM (before 2007) / or studies with more than 50 IM or more than 2 PM (after 2007)
  - either studies or case reports in which an alternative is suggested for treatment of IM and/or PM (lower dose or different drug) / or in which 6-TGN concentrations and doses are stated for NM and IM and/or PM / or in which the dose for IM was reduced to such an extent that there is no longer a difference in adverse events between NM and IM

  Following this selection, there were twenty articles prior to 2007, to which the following three articles were added on the advice of Dr L.J.J. Derijks:
  - Black AJ et al. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. Annals of Internal Medicine 1998;129:716-8.
  - Campbell S et al. Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low-dose azathioprine. Aliment Pharmacol Ther 2002;16:389-98.
  - Gearry RB et al. Thiopurine S-methyltransferase (TPMT) genotype does not predict adverse drug reactions to thiopurine drugs in patients with inflammatory bowel disease. Aliment Pharmacol Ther 2003;18:395-400.

  For the period after 2010:
  - clinical studies of patients with conditions other than auto-immune hepatitis, and not investigating genotype-guided therapy, were not included if the number of patients was lower than 600 (period from 2011 up to May 2015) or 750 (period from May 2015). These studies do not contribute sufficiently to the burden of proof.

    The article “Lennard L et al. Thiopurine methyltransferase genotype-phenotype discordance and thiopurine active metabolite formation in childhood acute lymphoblastic leukaemia. Br J Clin Pharmacol 2013;76:125-36.” was not included, because this is a less expansive version of Lennard Br J Haematol 2015;169:228-40 and contains no additional relevant information. The article “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.” was not included, because this is a less expansive version of Liu 2017 and contains no additional relevant information.

    The article “Booth RA et al. Assessment of thiopurine methyltransferase activity in patients prescribed azathioprine or other thiopurine-based drugs. Evid Rep Technol Assess (Full Rep) 2010;196:1-282.” was not included, because this contains a less expansive meta-analysis than Booth, 2011.
  - during the period from 2011 up to May 2015, there were no studies that examined the link between the TPMT genotype and measures of outcome in patients with auto-immune hepatitis
  - as the dose data for PM are limited, articles were also included in which a dose for PM was determined
  - kinetic studies were only included if they contained average doses corrected for 6-TGN concentrations per genotype group.
- <u>Dose recommendations in reviews/articles</u>
  - Clinical Pharmacogenetics Implementation Consortium Guidelines (Relling et al., Clin Pharmacol Ther 2011;89:387-91, Clin Pharmacol Ther 2013;93:324-5 and Clin Pharmacol Ther 2019;105:1095-1105. PubMed PMID: 30447069):

    CPIC defines TPMT IM and TPMT PM as we do (one or two no function alleles, respectively), but considers only *2, *3A, *3B, *3C, *4, *11, *14, *15, *23, and *29 to be no function alleles. CPIC considers the other alleles, including *5 through *10 and *12 to be alleles with uncertain function. CPIC groups combinations of one allele with uncertain function and one no function allele in the phenotype ‘possible IM’ instead of in the IM phenotype. In addition, CPIC groups combinations of two uncertain function alleles and combinations of one normal function and one uncertain function allele in the phenotype ‘indeterminate’.

    CPIC indicates that TPMT PM are at very high risk for life-threatening myelosuppression, due to very high 6-TGN levels, if given conventional doses of 6-mercaptopurine (or azathioprine). In addition, CPIC indicates that despite having higher 6-TGN levels than NM, only about 30–60% of TPMT IM cannot tolerate full doses of 6-mercaptopurine or azathioprine (Relling 1999, Evans 2001, 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 and 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). CPIC indicates that good thiopurine tolerance in some IM may be because, although they have higher 6-TGN levels than NM, they have lower concentrations (and, thus, fewer toxic effects) of the methylmercaptopurine nucleotides (6-

MMPN) than do NM, which may offset the toxic effects of having higher 6-TGN levels. Thus, there is less of a consensus over how to dose azathioprine and mercaptopurine in patients who are TPMT IM compared with those who are PM, although they are at a higher risk for toxicity compared with NM (Higgs 2010).

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

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

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

CPIC indicates that some of the clinical data upon which dosing recommendations are based rely on measures of TPMT phenotype rather than genotype; however, because TPMT genotype is strongly linked to TPMT phenotype (Schaeffeler E et al. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenetics 2004;14:407-17; Yates CR et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med 1997;126:608-14; Liu 2017; and Tamm R et al. Polymorphic variation in TPMT is the principal determinant of TPMT phenotype: a meta-analysis of three genome-wide association studies. Clin Pharmacol Ther 2017;101:684-95), these recommendations apply regardless of the method used to assess TPMT status.

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 TPMT phenotype			
Phenotype	Therapeutic recommendation		Classification of recommendation
	6-mercaptopurine	azathioprine	
IM (one no function allele: *2, *3A, *3B,	Start with reduced starting doses (30-80% of normal dose) if normal starting dose[a] is ≥ 75	Start with reduced starting doses (30-80% of normal dose) if normal starting dose[a] is 2-3	Strong[d]

*3C, *4, *11, *14, *15, *23, or *29) or possible IM (one allele with uncertain function (allele other than *1, *2, *3A, *3B, *3C, *4, *11, *14, *15, *23, or *29) and one no function allele)	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.	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].	
PM (two no function alleles: *2, *3A, *3B, *3C, *4, *11, *14, *15, *23, or *29)	For malignancy, start with drastically reduced doses (reduce daily dose[a] by 10-fold and reduce frequency to thrice weekly instead of daily (e.g., 10 mg/m^2/day given just 3 days/week) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Allow 4-6 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing 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 malignancy, start with drastically reduced doses (reduce daily dose[a] by 10-fold and dose thrice weekly instead of daily) 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. J Clin Pathol 2010;63:288-95; Stocco G et al. Clin Pharmacol Ther 2009;85:164-72; Lennard L et al. Ther Drug Monit 1996;18:328-34; Schmiegelow K et al. Leukemia 2009;23:557-64; Schmiegelow K et al. Leukemia 2010;24:345-54; Relling MV et al. Blood 2006;107:843-4; Sandborn WJ. 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. J Clin Pathol 2010;63:288-95; Sandborn WJ. 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.

[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. J Clin Pathol 2010;63:288-95; Evans WE et al. J Pediatr 1991;119:985-9; Sandborn WJ. 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. Lancet 2006;367:839-46; Sandborn WJ. 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 2003.

Recommendations for patients having also a NUDT15 variant

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

pound intermediate metabolisers remains limited.
The therapeutic recommendations for patients having also a NUDT15 variant are indicated below:

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

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

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

As evidence linking TPMT genotype with 6-mercaptopurine and/or azathioprine phenotype, CPIC mentions 128 articles. 104 of these articles were not included in our risk analysis. 13 articles were not included in our risk analysis because they concerned *in vitro* or preclinical (mouse) studies. The other not included studies also did not fulfil our inclusion criteria (see the first item under Comments). In addition, our risk analysis includes 22 articles not included by CPIC of which 3 were recent (i.e. published after the last CPIC search) (Fan 2019, Choi 2019, Eriksen 2017, Van Moorsel 2017, Kim 2012, Newman 2011, the systematic review (PM) and meta-analysis (IM) Higgs 2010, Hindorf 2010, Sheffield 2009, Ansari 2008, Gardiner 2006, Moloney 2006, Jun 2005, Kurzawski Ther Drug Monitor 2005, Kurzawski Transplant Int 2005, Fabre 2004, Gilissen 2004, Gearry 2003, Schaeffeler 2003, Campbell 2002, Langley 2002, and Pandy 2002). Instead of some of these articles, CPIC included other articles by the same author or group. CPIC indicates that the included *in vitro* studies provide a high level of evidence for mercaptopurine catabolism to methylmercaptopurine being absent in human erythrocytes, lymphocytes, liver, and kidneys from TPMT PM (4 studies), for the mechanisms of functional inactivation for TPMT *2, *3A, *3B, *3C, *4 demonstrated by expression of specific variant alleles (3 studies), for heterologous expression of TPMT catabolizing mercaptopurine to methylmercaptopurine, and TIMP (the 6-TGN precursor thioinosine monophosphate) to methylTIMP (2 studies), and for a higher sensitivity of TPMT knock-down cells to mercaptopurine in some cases compared to wild type (1 study). One *in vitro* study provided a low level of evidence that TPMT deficiency could lead to chronic exposure to thiopurine and could be linked to development of brain cancer (astrocytomas). CPIC indicates that the preclinical studies provide a high level of evidence for TPMT+/+ mice having higher survival with high doses of mercaptopurine but TPMT-/- mice having improved survival with lower doses (1 study), and for TPMT knock-out mice having more morbidity and mortality but better ALL efficacy from mercaptopurine than wild type mice; heterozygotes were at intermediate risk (2 studies), CPIC indicates that clinical studies provide a high level of evidence for increased risk of myelosuppression in TPMT IM receiving normal doses of mercaptopurine or azathioprine (43 references including Black 1998, McLeod 1999, Relling 1999, Colombel 2000, Evans 2001, Zelinkova 2006, the meta-analysis of Booth 2011, Lee 2013, Belen 2014, and Kim 2014), for TPMT status to be associated with dose reduction or cessation of therapy of azathioprine or mercaptopurine (17 references, including Evans 2001, Kaskas 2003, Lee 2013, Kim 2014, Lennard Br J Haematol 2015;169:228-40, and Liu 2017), for personalized dose for TPMT variant genotypes being significantly associated with decreased hematologic adverse drug reaction risk and decreased 6-TGN levels compared with standard doses (Coenen 2015), for TPMT wild-type patients with ALL having higher risk of relapse than those with at least one variant TPMT allele, particularly in regimens that are primarily antimetabolite-based, and wild-type patients with IBD having higher risk of treatment failure (4 references, including Ansari 2002), for TPMT PM having life-threatening toxicity (myelosuppression) from normal doses of mercaptopurine and azathioprine; toxicity can be minimized with substantially decreased doses (14 references, including Black 1998, McLeod 1999, Relling 1999, Colombel 2000, Kaskas 2003, and Zelinkova 2006), for increased risk of leukopenia in TPMT IM and PM receiving thiopurines for treatment of chronic inflammatory diseases (the meta-analysis of Booth 2011), for higher level of residual leukemia in TPMT NM than in IM/PM with ALL after 10 days of fixed-dose thiopurine but not in absence of thiopurines (Stanulla 2005), for no increase in acute toxicity in IM compared to NM with ALL who received mercaptopurine doses adjusted downward for TPMT defective patients (3 references, including Evans 2001), for TPMT genotyping to be useful in predicting myelosuppression from azathioprine in transplant recipients (5 references, none of which included in our risk analysis), and for no change in treatment efficacy for IBD patients who receive azathioprine based on TPMT status or thioguanine concentration (1 reference that is not included

in our risk analysis). In addition, CPIC reports a high level of evidence that TPMT genotype correlates with TPMT activity measured by biochemical assay (variant genotypes have lower activity in general than *1/*1), but activity cannot be explained by genotype alone because the *1/*1 and variant (heterozygote) activities overlap (17 references, including Relling 1999, Ansari 2002, the meta-analysis of Booth 2011, Demlova 2014, and Liu 2017), and that TPMT variant genotype is associated with increased TGN levels and/or lower methylmercaptopurine nucleotide levels (8 references, including Kim 2014). CPIC reports a moderate level of evidence that *3C variant is associated with alopecia in patients with autoimmune disease (i.e. inflammatory bowel disease and lupus) (2 references, including Kim 2014), that TPMT variant genotype is NOT associated with greater likelihood of event free survival, but studies that adjust dose based on TPMT status or tolerance may be unlikely to find such associations (6 references, including Levinsen 2014, Lennard Br J Haematol 2015;169:228-40, and Lennard Br J Haematol 2015;170:550-8), that there is no change in relapse risk for IM with ALL who receive mercaptopurine doses adjusted downward for TPMT defective patients (2 references, both not included in our risk analysis), that risk of secondary leukemia in those with low TPMT activity and in those with high thiopurine active metabolites is increased (7 references, including Levinsen 2014), that TPMT genotyping is useful in predicting myelosuppression and likelihood of clinical response to azathioprine/mercaptopurine in IBD (11 references, including Zelinkova 2006 and Gardiner 2008), that TPMT genotyping is useful in predicting myelosuppression and likelihood of clinical response to azathioprine in Crohn's disease (5 references, including Colombel 2000 and Gardiner 2008), that TPMT genotype-based dosing reduced toxicity while maintaining drug efficacy in trial of azathioprine for moderate-severe atopic eczema (1 reference, published before 2010 and not included in our risk analysis), that TPMT genotyping is useful in predicting myelosuppression from azathioprine in rheumatoid arthritis (4 references, none of which included in our risk analysis), and that risk of hepatotoxicity to mercaptopurine in patients with TPMT wild-type genotype and/or higher mercaptopurine metabolites (6-MMPN (6-methylmercaptopurine nucleotides)) is increased (8 references, none of which included in our risk analysis). CPIC reports a weak level of evidence that TPMT variant genotype is associated with incidence of gastrointestinal adverse drug reactions (4 references, of which none included in our risk analysis), that TPMT status is associated with development of secondary cancer (5 references, including Levinsen 2014 and Lennard Br J Haematol 2015;169:228-40), and that the VNTR (variable number of tandem repeats) region in the TPMT promoter correlates with TPMT expression (not statistically significant) (1 reference that is not included in our risk analysis). CPIC also reports a weak level of evidence that TPMT status is associated with development of secondary cancer based on 7 references, none of which are included in our risk analysis, but this seems to be a mistake, because this outcome is reported with other references directly above and not all of the 7 references mentioned concern cancer patients.

On 14-8-2019, there was not a more recent version of the recommendations present on the CPIC-site.

- Review Smith et al. (Pharmacogenomics 2010;11:421-37): recommendation is to treat PM with 5-8% of the azathioprine dose used for NM and to treat IM with 50-60% of the dose used for NM.
- Schmiegelow et al. (Leukemia 2009;23:557-64): Due to their higher risk of myelosuppression and supposed risk of secondary malignant tumours, the Scandinavian Association for Paediatric Haematology and Oncology (NOPHO) has, since 2001, adjusted the initial dose of mercaptopurine according to the TPMT genotype in all its ALL protocols (IM: 66.7% of the dose for NM (75 mg/m^2), PM: 13.3% of the dose for NM). The dose is then adjusted according to the leukocyte count. It is not yet known whether this strategy affects the frequency of relapse of ALL for IM and PM. The old protocol found a lower risk of relapse of ALL for patients with low TPMT activity compared to patients with normal TPMT activity (7% versus 18%), but no improved survival, possibly due to a higher frequency of secondary tumours in this patient group.
- Review Wang and Weinshilboum (Oncogene 2006;25:1629-1638): In order to avoid toxicity, homozygous polymorphic patients should be treated with 1/10th to 1/15th of the standard dose of thiopurine and even then they require careful monitoring.

- <u>Cost-effectiveness</u>:
  - Zarca K et al. Modeling the outcome of systematic TPMT genotyping or phenotyping before azathioprine prescription: a cost-effectiveness analysis. Mol Diagn Ther 2019;23:429-38. PubMed PMID: 30963516. The additional costs per case of severe myelosuppression averted, were calculated for French adult patients with IBD for whom azathioprine was considered suitable as first-line monotherapy. 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 hospitalization. Screening for TPMT deficiency, with either genotyping or phenotyping, was compared to the absence of screening. The additional costs per case of severe myelotoxicity averted were € 2,602 in the phenotyping strategy, and € 11,244 in the genotyping strategy compared to the no screening strategy. At prevalence rates of severe myelotoxicity > 1%, phenotyping was both cheaper and better than genotyping and no screening strategies. The probability of phenotyping to be cost-effective was 90% if the decision-maker is willing to pay more than € 7000 (median cost for a hospital stay for toxic myelosuppression) for an additional averted episode of severe myelotoxicity. However, because the additional costs for averting one case of severe myelotoxicity were considerably larger than the median costs of treating one case

of severe myelotoxicity, genotyping was unlikely to be cost-effective.
Genetic screening was for TPMT*2, *3A, *3B and *3C. Patients with TPMT deficiency (TPMT PM or very low TPMT activity) 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 payer perspective. Only direct medical costs (costs of the diagnostic test and costs of treatment) were included in the calculation. The mean estimated costs of the no screening, phenotyping and genotyping strategies for 1 year were € 409/patient, € 427/patient and € 476/patient, respectively. Phenotyping resulted in 0.007 severe myelosuppressions avoided over a year versus 0.006 for genotyping. Costs for azathioprine (including monthly cell blood counts and liver tests) were € 300/year, costs for TNF-α inhibitors (including office visits, and hospitalisations for infusion) were € 4200/year, TPMT genotyping costs were € 110 (corresponding to the reimbursement by the health care system), and TPMT enzymatic activity assay costs were € 67 (corresponding to the reimbursement by the health care system). Prevalence rates of TPMT genotypes/phenotypes, sensitivity and specificity of TPMT testing, and incidence of severe myelosuppression from previous reports were used. From these data, the authors selected the most frequently reported values: a prevalence of TPMT PM of 0.6% and a prevalence of low TPMT activity in the general population of 0.7%, a sensitivity of genotyping of 60% and a sensitivity of phenotyping of 70%, a specificity of genotyping of 99.6% and a specificity of phenotyping of 99.7%, and a probability of a severe myelotoxicity of 1%. The authors assumed that 100% of those with low TPMT activity 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.

- Plumpton CO et al. A systematic review of economic evaluations of pharmacogenetic testing for prevention of adverse drug reactions. Pharmacoeconomics 2016;34:771-93. PubMed PMID: 26984520.
The authors performed a systematic literature review of economic evaluations of pharmacogenetic tests of TPMT prior to prescription of 6-mercaptopurine or azathioprine. The authors conclude that economic evidence was inconclusive, with considerable variation in results across several high-quality studies indicating that genotyping was not cost effective. The authors mentioned that there was only one notable trial of TPMT genotyping preceding start of 6-mercaptopurine or azathioprine (Newman 2011).
Eleven economic evaluations were retrieved: four conducted in Canada (Donnan 2011, Marra 2002, Sayani FA et al. Thiopurine methyltransferase enzyme activity determination before treatment of inflammatory bowel disease with azathioprine: effect on cost and adverse events. Can J Gastroenterol 2005;19: 147-51, and Tavadia 2000), two conducted in the USA (Dubinsky 2005 and Hagaman 2010), two conducted in the UK (Thompson 2014 and Winter 2004), one conducted in Europe (other than the UK) (Van den Akker-van Marle 2006), one in New Zealand (Priest 2006), and one in Korea (Oh KT et al. Pharmacoeconomic analysis of thiopurine methyltransferase polymorphism screening by polymerase chain reaction for treatment with azathioprine in Korea. Rheumatology 2004;43:156-63). Four studies were cost-minimisation or cost-benefit analyses (Donnan 2011, Dubinsky 2005, Sayani 2005, and Tavadia 2000). Four studies were cost-effectiveness analyses reporting costs per life-year gained (van den Akker-van Marle 2006 and Winter 2004) or events averted (Marra 2002 and Oh 2004). Three evaluations were cost-utility analyses *(*Thompson 2014, Hagaman 2010, and Priest 2006). Costs were calculated from the perspective of the healthcare provider in four studies (Thompson 2014, Donnan 2011, Sayani 2005, and Winter 2004) and (also) from a societal perspective in four (van den Akker-van Marle 2006, Oh 2004, Winter 2004, and Marra 2002). Eight evaluations (Donnan 2011, Hagaman 2010, Priest 2006, van den Akker-van Marle 2006, Dubinsky 2005, Oh 2004, Winter 2004, and Marra 2002) were based on economic models, two were conducted alongside prospective randomised studies (Thompson 2014 and Sayani 2005) and one analysis was based on a case report (Tavadia 2000). The quality of reporting in the economic evaluations was high for 7 of the 11 studies (Thompson 2014, Donnan 2011, Priest 2006, Dubinsky 2005, Sayani 2005, Oh 2004, and Marra 2002). High quality was defined as reporting of more than 85% of items on a 24-item checklist for economic health evaluations. The perspective was unclear in Tavadia 2000. Van den Akker-van Marle 2006 did not state explicitly the modelling approach. Van den Akker-van Marle 2006 and Winter 2004 did not mention explicitly a time horizon, although a lifetime time horizon could be assumed for these studies. Winter 2004 did not mention sensitivity analysis explicitly. Four studies stated that the evidence supporting the effectiveness of pharmacogenetics testing was retrieved from literature searches (Donnan 2011, Dubinsky 2005, Oh 2004, and Marra 2002), two mentioned retrospective genotyping as source (Priest 2006 and Winter 2004), one mentioned cohort studies (Hagaman 2010), and two were vague about the sources (Tavadia 2000, Van den Akker-van Marle 2006). Two studies used random controlled trial evidence of the effectiveness of pharmacogenetics testing (Thompson 2014 and Sayani 2005).
Studies had mixed results across different settings and disease areas. While four high-quality studies found testing to be cost saving or to be both cost saving and better than standard dosing (Donnan 2011, Dubinsky 2005, Oh 2004, and Marra 2002), evaluations based on random controlled trial evidence indica-

ted that testing was either not cost effective (Sayani 2005) or was less costly and less effective (Thompson 2014). Phenotype testing was less expensive (Donnan 2011) or more cost effective (Priest 2006) than genotyping, and this is more in line with practice (Thompson 2014). The authors state that the FDA recommends genetic testing before prescribing azathioprine, whilst the Japanese PMDA (Pharmaceuticals and Medical Devices Agency) and Health Canada (Sante Canada; HCSC) both note that there are actionable genetic variants that determine efficacy, dosage or toxicity.

- Thompson et al. (Value Health 2014;17:22-33. PubMed PMID: 24438714): The authors use a randomised study in which 167 patients with auto-immune diseases with TPMT genotype known before start of azathioprine were compared to 166 patients for who this was not the case (Newman 2011). The data from this study and costs of £ 20 per genotyping test did not result in significantly reduced costs for genotype-based treatment (- £ 421; 95% CI: -925 – 90) and the quality-adjusted life-years gained decreased very little and non-significantly (-0.008 years over a follow-up period of 4 months; 95% CI: -0.017-0.0002). At a sum of £ 20,000 per life-year gained, the net benefits (amount per life-year gained x difference in life-years gained minus the difference in costs) were not significantly positive (£ 257; 95% CI: -426 – 933). At TPMT test costs of £ 350, equivalent to a “medium genetic test” from a 2006 consultation for a national tariff for genetic tests, genotyping would have only a 47% chance of being cost-effective at a threshold of £ 20,000 per life-year gained. If the test instead costs only £ 150, the price of a “simple genetic test”, then the probability of TPMT genotyping being cost-effective would rise to 71%. With Newman 2011 not demonstrating a better overall outcome for genotype-guided versus non-genotype-guided therapy, this probability of cost-effectiveness is due to the lower medication costs for IM receiving reduced azathioprine dose.
  Doctors were advised to use an initial dose of 2-3 mg/kg per day for NM. A reduced initial dose was recommended for IM (for example 25-50 mg/day), followed by titration of the maintenance dose. PM were not present in the group for which the genotype was known at the start of treatment. The primary endpoint of the study was withdrawal of azathioprine due to adverse events in the first four months of treatment. There was no difference in the primary endpoint between the groups with genotype-based and non-genotype-based treatment. There was also no difference in the primary endpoint between NM and IM in the group with genotype-based treatment. In this group, the initial dose for IM was lower than for NM, but there was no difference in the dose after 4 months. However, the initial dose for NM with genotype-based treatment was equal to the initial dose for the non-genotype-based treatment and 2-3x lower than the recommended initial treatment. There was little difference in the use of healthcare services between the groups with genotype-based and non-genotype-based treatment. The number of patients with more than 1 hospital admission was lower in the genotyping group (0.6% versus 3.6%). At £ 20 per genotyping test, this resulted in lower, calculated costs for genotype-based treatment (£ 1,683.40 versus £ 1,966.78). However, the difference in costs was not significant after correction (- £ 421; 95% CI: -925 – 90). Quality-adjusted life-years (QALYs) were calculated from the health-related quality of life measured before and after treatment. The number of QALYs increased less in the genotyping group (0.233 versus 0.243). However, the difference was not significant after correction (-0.008; 95% CI: -0.017 – 0.0002). The probability of cost-effectiveness decreased as the costs per life-year gained increased and as the costs for the genotyping test increased.
- Donnan JR et al. (Pediatr Blood Cancer 2011;57:231-9): Genotyping for TPMT was not cost-effective in the treatment of patients with acute lymphoblastic leukaemia with mercaptopurine. The costs increased with genotyping by 277 Canadian dollars (95% CI: 112-442), whilst the overall survival did not increase in the first three months of the treatment. Cost-effectiveness was determined in comparison to a standard treatment, where the initial dose was calculated according to weight. However, if myelosuppression occurred, the genotype was determined and the desired dose reduction was determined accordingly. The genotype-based treatment was based on the standard treatment for NM and IM (dose adjustment only in the case of myelosuppression). A reduction of the initial dose was only assumed for PM. The difference between genotype-based and non-genotype-based treatment is therefore small.
  The parameters used in the model were obtained from the literature or based on expert opinion. Parameters included genotyping costs of $ 460 per test, a 3% incidence of myelosuppression, hospital admission in 15% of the cases of myelosuppression and a mortality risk of 2.9% following hospital admission for febrile neutropenia and 0% following other forms of myelosuppression. It was assumed that 30% of the cases of myelosuppression were prevented by dose reduction in IM and PM. Considering the high percentage of myelosuppression in PM at initial dose, this is probably an underestimate for PM. Genotyping would be cost-effective at genotyping costs below $ 12 per test.
  The authors reported that the Children's Oncology Group in 2008 recommended an initial dose of 60% for IM and less than 10% for PM. The Children's Oncology Group recommended no genotyping before start of treatment, but only after occurrence of adverse events in order to determine the desired dose reduction.
- Hagaman et al. (Lung 2010;188:125-32): The authors estimate that TPMT genotyping before start of thiopurine therapy in patients with pulmonary fibrosis reduces the absolute risk of developing azathioprine-related bone marrow toxicity by approximately 2%. As approximately 16% of the patients with azathioprine-related leukopenia require hospital admission, this means that approximately 50

patients need to be screened in order to prevent one case of leukopenia and approximately 313 patients need to be screened to prevent one hospital admission. As TPMT genotyping is relatively cheap, the prevention of one case of complicated leukopenia should easily outweigh the costs of the tests.

- Review Compagni et al. (Int J Technol Assess Health Care 2008;24:294-302): The authors concluded that TPMT genotyping is not cost-effective at a price of € 68 for a genotyping assay. At this price, the costs of a case of neutropenia amount to € 2,116 and the costs of preventing a case of neutropenia amount to € 5,300.
- Review Teml et al. (Clin Pharmacokinet 2007;46:187-208): Dubinsky 2005 found that TPMT genotyping before start of treatment reduces both the time to response for azathioprine (19.1 versus 22.4 weeks) and the treatment costs ($ 3861 versus $ 7142) in patients with Crohn's disease and ulcerative colitis (Dubinsky MC et al. A cost-effectiveness analysis of alternative disease management strategies in patients with Crohn's disease treated with azathioprine or 6-mercaptopurine. Am J Gastroenterol 2005;100:2239-47). Similar results were found by Winter 2004 and Priest 2006 (Winter J et al. Cost-effectiveness of thiopurine methyltransferase genotype screening in patients about to commence azathioprine therapy for treatment of inflammatory bowel disease. Aliment Pharmacol Ther 2004;20:593–9, and Priest VL et al. Pharmacoeconomic analyses of azathioprine, methotrexate and prospective pharmacogenetic testing for the management of inflammatory bowel disease. Pharmacoeconomics 2006;24:767-81). Cost-effectiveness was also found in studies that examined the effect of genotyping for TPMT with thiopurine treatment for ALL (Van den Akker-van Marle 2006) or arthritic or dermatological conditions (Marra 2002 and Tavadia 2000) (van den Akker-van Marle ME et al. Cost-effectiveness of pharmacogenomics in clinical practice: a case study of thiopurine methyltransferase genotyping in acute lymphoblastic leukemia in Europe. Pharmacogenomics 2006;7:783–92, Marra CA et al. Practical pharmacogenetics: the cost effectiveness of screening for thiopurine s-methyltransferase polymorphisms in patients with rheumatological conditions treated with azathioprine. J Rheumatol 2002;29:2507-12, and Tavadia SM et al. Screening for azathioprine toxicity: a pharmacoeconomic analysis based on a target case. J Am Acad Dermatol 2000;42:628-32). One study (29 patients with Crohn's disease or ulcerative colitis) found higher costs (primarily due to the costs of the genotyping assay) if genotyping was performed, but there was only one patient in this small study who developed TPMT-related toxicity. A large, prospective study into cost-effectiveness of TPMT genotyping in patients with Crohn's disease or ulcerative colitis was started in 2007.

Date of literature search: 12 June 2019.

	Phenotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	PM	4F	Yes	Yes	17 September 2019
	IM	4E	Yes	Yes	

N.B. Some articles refer to a lower efficacy for NM with relatively high TPMT activity (called "high NM" in the risk analysis). This could correspond to a UM, but has not been defined (yet) for TPMT. The working group has decided to only provide therapeutic recommendations based on genotype (i.e. for IM and PM).

Mechanism:

Lower metabolic activity of TPMT leads to increased intracellular concentrations of thioguanine nucleotides, the active metabolites of azathioprine and mercaptopurine. This increases the risk of adverse events such as myelosuppression.

Azathioprine and mercaptopurine are inactive pro-drugs, which are converted to the active metabolites – thioguanine nucleotides - in the body via several steps. Azathioprine is converted in the body to mercaptopurine and nitromethyl imidazole. 6-Mercaptopurine is then converted to thioguanine nucleotides in three steps. The first of these steps is catalysed by the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT).

Two catabolic routes reduce mercaptopurine bio-availability for thioguanine nucleotide formation. Thiopurine methyltransferase (TPMT) catalyses S-methylation of both mercaptopurine and the metabolites formed by HPRT (6-mercaptopurine nucleotides or 6-thio-inosine nucleotides). The methylated 6-thio-inosine nucleotides contribute to the anti-proliferative properties of the thiopurines, probably through inhibition of *de novo* purine synthesis. High concentrations of methylated 6-thio-inosine nucleotides are also associated with a higher risk of hepatotoxicity. In addition to this, mercaptopurine is oxidised to the inactive 6-thiouric acid by the enzyme xanthine oxidase (XO), which occurs primarily in the liver and intestines.

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