NUDT15: thioguanine 7033/7034

ALL = acute lymphoblastic leukaemia, EM = extensive metaboliser (*1/*1) (normal NUDT15 enzyme activity), IM = intermediate metaboliser (*1/*2, *1/*3, *1/*4, *1/*5 *1/*6) (reduced NUDT15 enzyme activity), NUDT15 = nudix hydroxylase 15 (enzyme involved in hydrolysis of diphosphate bonds and thus the conversion of 6-thio-deoxyguanosine triphosphate (6-thio-dGTP) into 6-deoxythioguanosine monophosphate (6-thio-dGMP)), PM = poor metaboliser (e.g. *2/*2, *2/*3, *3/*3) (absent or strongly reduced NUDT15 enzyme activity), 6-TGN = 6-thioguanine nucleotides (the active metabolites of azathioprine/6-mercaptopurine), 6-thio-dGTP = 6-thio-deoxyguanosine triphosphate (the fully activated metabolite of azathioprine/6-mercaptopurine), TPMT = thiopurine S-methyltransferase (enzyme involved in formation of inactive thiopurine metabolites).

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

Brief summary and justification of choices:
NUDT15 reverses the final step in the formation of the active metabolite of thioguanine. It converts 6-thiodeoxyguanosine triphosphate (6-thio-dGTP), which is incorporated into DNA, into 6-thiodeoxyguanosine monophosphate (6-thio-dGMP). For this reason, lower metabolic activity of NUDT15 leads to increased intracellular concentrations of the active metabolite 6-thio-dGTP. This increases the risk of adverse events such as myelosuppression.
There were no studies in humans confirming an increased thioguanine toxicity risk in patients with a genetically reduced NUDT15 enzyme activity (intermediate metabolisers (IM) and poor metabolisers (PM)). However, for azathioprine and mercaptopurine, which have the same active metabolite as thioguanine, nine studies with more than 175 patients and two meta-analyses confirm IM and PM to have an increased risk for leukopenia and/or dose reduction due to adverse events like myelosuppression and infection. In addition, the thioguanine concentration killing 50% of acute lymphoblastic leukaemia blasts *in vitro* was significantly lower for cells derived from IM patients than for cells derived from EM patients (Moriyama T et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet 2016;48:367-73. PubMed PMID: 26878724). For these reasons, the KNMP pharmacogenetics working group decided that it was justified to extrapolate the data for azathioprine and 6-mercaptopurine to thioguanine and decided that therapy adjustment is required for the NUDT15-thioguanine interactions (yes/yes-interactions).
Therapeutic recommendations
Because *in vivo* data for thioguanine are lacking and extrapolation from the data on either azathioprine and 6-mercaptopurine or TPMT phenotypes was decided to be justified, the therapeutic recommendations for thioguanine were based on the azathioprine and 6-mercaptopurine and/or TPMT data.
Because there is no assay for determining 6-thio-dGTP levels in patient cells, only maximum tolerated doses can be used to calculate dose adjustments for patients with NUDT15 variants. Five studies with more than 175 patients (Yi 2017, Kim 2017, Chao 2017, Liang 2016 and Yang 2015) and one meta-analysis (Yin 2017) report tolerated doses of azathioprine and/or 6-mercaptopurine (see the risk analysis for azathioprine and 6-mercaptopurine for full references and details). Calculations were only based on the five studies because the meta-analysis also includes two of the studies. In addition, the meta-analysis does not specify the criteria for inclusion of studies for dose calculations and also included the studies of Moriyama 2016 and Yang 2014, which do not provide clear data on tolerated doses. Four of the studies used for dose calculations concern acute lymphoblastic leukaemia (ALL) therapy (Yi 2017, Kim 2017, Chao 2017, Liang 2016 and Yang 2015). The fifth study concerns inflammatory bowel disease therapy, but provides only data for IM (Chao 2017).

PM: The weighted mean of the calculated dose adjustment for 13 PM from 4 ALL studies is a dose reduction to 20% (10-24%, median 20%). In ALL therapy, 6-mercaptopurine and methotrexate dose are usually lowered alternately in case of leukopenia or other side effects. This indicates that using 20% of the normal 6-mercaptopurine dose in combination with normal methotrexate doses most probably will still result in leukopenia and the need for dose adjustment. For this reason, the KNMP pharmacogenetics working group decided to recommend starting with the lower range of the observed tolerated doses, i.e. 10% of the normal dose. This would make the dosing recommendation the same as for TPMT PM. This corresponds to the similar effect of

variants of these two genes on the tolerated thiopurine dose as observed in two studies comparing NUDT15 and TPMT directly (Liang 2016 and Yang 2015).
Because the dose adjustment could not be calculated, but had to be extrapolated, the KNMP Pharmacogenetics Working Group decided to recommend adjusting the dose in second instance and choosing another drug in first instance.

IM: The weighted mean of the calculated dose adjustment for 283 IM from 5 studies is a dose reduction to 71% (65-78%, median 70%). In ALL therapy, 6-mercaptopurine and methotrexate dose are usually lowered alternately in case of leukopenia or other side effects. In addition, in the only inflammatory bowel disease study, the tolerated dose was only calculated in patients tolerating azathioprine or 6-mercaptopurine for more than 6 months (60% of the EM, 47% of the IM and none of the PM). This suggests that in this study, the patients most sensitive to thiopurines were not included in the calculation. This indicates that using 70% of the normal 6-mercaptopurine dose either in combination with normal methotrexate doses or in the more sensitive halve of IM patients most probably will still result in leukopenia and the need for dose adjustment. However, two studies comparing NUDT15 and TPMT directly observed a similar effect of variants of these two genes on the tolerated thiopurine dose (Liang 2016 and Yang 2015). Because TPMT variants do not affect the ratio of the different 6-TGNs, the dose adjustment for TPMT IM could also be calculated based on the change in total 6-TGN level. A study including 40 TPMT IM patients showed that the median concentration of the active metabolite of thioguanine was 30% higher compared to EM patients, despite equal median doses (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). This is equivalent to a dose reduction to 77% to achieve the same median concentration of the active metabolite in IM patients as in EM patients at the standard dose. This was rounded off to 75% to be more achievable in clinical practice. Because thioguanine is often used as a last resort, the KNMP Pharmacogenetics Working Group decided to recommend a starting dose of 75% of the normal dose despite the limited evidence.
For oncolytics, toxicity and efficacy are strongly coupled, and it is unknown whether starting with a dose reduction based on genotype results in the same efficacy as reducing the dose based on toxicity. For this reason and because some TPMT IM tolerate the full dose, the KNMP Pharmacogenetics Working Group recommendation for these patients is to either start with 75% of the normal thioguanine dose or to start with the normal dose and reduce to 75% when adverse events necessitate a dose reduction. In determining the starting dose, next to the IM phenotype, the physician needs to take into account the comorbidity (e.g. the sensitivity for infections), the patient wishes (taking into account the above mentioned uncertainty) and the estimation of the aggression of the tumour (e.g. based on tumour genetics).

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

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

The Dutch Pharmacogenetics Working Group considers genotyping before starting thioguanine to be essential for drug safety. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection.

Data in humans are lacking for thioguanine, but extrapolation of data from azathioprine and 6-mercaptopurine was considered to be justified (see Brief summary and justification of choices above). For this reason, for determination of the clinical implication score for thioguanine, the severity of the clinical effect, the evidence supporting this severity and the percentage of patients with leukopenia due to a NUDT15 variant were derived from azathioprine/6-mercaptopurine. This resulted in the clinical implication of the NUDT15-thioguanine interaction scoring 6 out of the maximum of 10 points for patients of European and African descend and 7 out of the maximum of 10 points for patients of Asian or (Latin-)American descend (with pre-emptive genotyping considered to be essential for scores ranging from 6 to 10 points):

The risk of serious life-threatening toxicity of azathioprine and 6-mercaptopurine (code E corresponding to grade 4) is increased for patients with a genotype resulting in diminished NUDT15 enzyme activity (IM and PM). This results in 1 out of the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (1 point for CTCAE grade 3 or 4).

The increased risk for serious toxicity (code D-E corresponding to grade 3-4) has been shown in 9 studies and 2 meta-analyses on azathioprine and 6-mercaptopurine. This results in the maximum score of 3 points for the second criterion of the clinical implication score, the level of evidence supporting the associated clinical effect grade ≥ 3 (3 points for three or more publications with level of evidence score ≥ 3).

The number needed to genotype was deduced from the increase in the percentage of IM patients with azathioprine/6-mercaptopurine induced leukopenia. IM is the most prevalent variant genotype. For IM, an additional 23-77% of patients developed leukopenia compared to EM (ref. 1, 2 and 5). The lower limit of 23% was used for the calculation. For Asians, Hispanics and Americans, the prevalence of IM has been reported to be 9.4-20.7%. This would amount to 2-5% of patients in these populations in which leukopenia is due to a NUDT15 variant and thus

could have been prevented by reducing the risk to that in EM by lowering the dose, i.e. a number needed to genotype of 20-50. For Europeans and Africans, the prevalence of IM has been reported to be 0.6-1.5%. This would amount to 0.14-0.35% of patients developing leukopenia due to a NUDT15 variant in these populations, i.e. a number needed to genotype of 300-700. The calculated number needed to genotype of 20-50 for Asians and (Latin-) Americans results in 2 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) to prevent one clinical effect grade ≥ 3 (2 points for 10 < NNG ≤ 100). The calculated number needed to genotype of 300-700 for Europeans and Africans results in 1 out of the maximum of 3 points (1 point for 100 < NNG ≤ 1000).
The Summary of Product Characteristics (SmPC) indicates that patients with a variant NUDT15 gene have an increased risk for severe toxicity of thioguanine, such as early leukopenia and alopecia, at conventional doses of thiopurine therapy. This results in 1 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (1 point for at least one genotype/phenotype mentioned in the SmPC, but not mentioned as a contra-indication and no recommendation to genotype).

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

Source	Code	Effect	Comments
ref. 1 SmPC Lanvis (6-thioguanine) 08-06-18.	0 IM: E PM: E	<u>Dosing</u>: Patients with an inherited mutated NUDT15 gene have an increased risk for severe toxicity of thioguanine. In general, these patients require a dose reduction, especially if they are homozygous for the NUDT15 variant. Genotypic testing for NUDT15 variants before starting thioguanine therapy can be considered. At least, careful monitoring of blood cell counts is necessary. <u>Warning</u>: Patients with an inherited mutated NUDT15 gene have an increased risk for severe toxicity of thioguanine, such as early leukopenia and alopecia, at conventional doses of thiopurine therapy. In general, these patients require a dose reduction, especially if they are homozygous for the NUDT15 variant. The frequency of NUDT15 c.415C>T has an ethnic variability of approximately 10% in East-Asians, 4% in Latin-Americans, 0.2% in Europeans and 0% in Africans. At least, careful monitoring of blood cell counts is necessary. <u>Pharmacodynamics</u>: Recent studies indicate a strong association between the NUDT15 variant NUDT15 c.415C>T [p.Arg139Cys] (also known as NUDT15 R139C [rs116855232]), which is believed to result in loss of function of the NUDT15 enzyme, and thiopurine mediated toxicity, such as leukopenia and alopecia. Patients homozygous for the NUDT15 variant (NUDT15 T risk alleles) have a very high risk of thiopurine toxicity compared to patients homozygous for C. The frequency of NUDT15 c.415C>T has an ethnic variability with an increased risk in the Asian and Latin-American population. Genotypic analysis of the NUDT15 genotype should be performed. The prescribing physician is advised to determine if decreasing the dose is necessary based on the genetic profile of the patient, eventually in combination with the profile of adverse events occurring during treatment. Patients with variants in both the NUDT15 and the TPMT enzyme have significantly less tolerance for thiopurines than patients with risk alleles of only one of these two genes. The exact mechanism of NUDT15 associated thiopurine-related toxicity is not clear.	

Risk group	TPMT IM or PM

Comments:

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National Cancer Institute Common Toxicity Criteria (NCI-CTC)

	grade 1 = B	grade 2 = C	grade 3 = D	grade 4 = E	grade 5 = F
Diarrhoea	Increased stool frequency by < 4; slight increase in stoma output	Increased stool frequency by 4-6; moderate increase in stoma output; no effect on daily activities	Increased stool frequency by ≥ 7; incontinence; IV fluid ≥ 24 hours; hospitalisation; severe increase in stoma output; effect on daily activities	Life-threatening consequences (e.g. haemodynamic collapse)	Death
Neutropenia	> $1.5x10^9$/L	< 1.5-$1.0x10^9$/L	< 1.0-$0.5x10^9$/L	< $0.5x10^9$/L	Death
Leukopenia	> $3.0x10^9$/L	< 3.0-$2.0x10^9$/L	< 2.0-$1.0x10^9$/L	< $1.0x10^9$/L	Death
Thrombocytopenia	> $75x10^9$/L	75-$50x10^9$/L	50-$25x10^9$/L	< $25x10^9$/L	Death
Febrile neutropenia	-	-	Present	Life-threatening consequences (e.g. septic shock, hypotension, acidosis, necrosis)	Death

ULN = upper limit of normal

Date of literature search: 9 July 2018.

	Phenotype	Code	Gene-drug interaction	Action	Date
Pharmacogenetics Working Group decision	IM	0 E	yes	yes	4 March 2019
	PM	0 E	yes	yes	

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

Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

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

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

Clinical Implication Score Criteria	Possible Score	Given Score	
		European, African	Asian, (Latin-) American
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)			

• CTCAE Grade 3 or 4 (clinical effect score D or E) • CTCAE Grade 5 (clinical effect score F)	+ ++	+	+
Level of evidence supporting the associated clinical effect grade ≥ 3 • One study with level of evidence score ≥ 3 • Two studies with level of evidence score ≥ 3 • Three or more studies with level of evidence score ≥ 3	 + ++ +++	 +++	 +++
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3 • 100 < NNG ≤ 1000 • 10 < NNG ≤ 100 • NNG ≤ 10	 + ++ +++	 +	 ++
PGx information in the Summary of Product Characteristics (SmPC) • At least one genotype/phenotype mentioned OR • Recommendation to genotype OR • At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	 + ++ ++	 +	 +
Total Score:	10+	6+	7+
Corresponding Clinical Implication Score:		Essential	Essential