Supplementary material 1

Demographic distribution of the population under study; age and TPMT genotype distribution of the subjects. The overall number of subjects is 267 involving 229 patients with suspected haematological malignancies, 16 patients with suspected inflammatory bowel disease or other, mostly autoimmune disorders, and 22 blinded paediatric patient samples (not included in the table) used for method validation.

	Wildtype patients	**Heterozygote patients**	**Compound heterozygote patients**	**All patients**
Patients with suspected and/or confirmed hematological malignancy				
Children, adolescent [0-20]				
Number of female subjects	81	11	0	92
Median female age (years[range])	10 [0-20]	5.5 [2-19]	0 [0-0]	9 [0-20]
Number of male subjects	112	13	1	126
Median male age (years[range])	12 [0-19]	8 [1-18]	14	11 [0-19]
Adult [21-99]				
Number of female subjects	3	1	0	4
Median female age (years[range])	73 [56-78]	36	0 [0-0]	64.5 [36-78]
Number of male subjects	5	2	0	7
Median male age (years[range])	65 [34-93]	59 [50-68]	0 [0-0]	65 [34-93]
IBD patients				
Number of female subjects	5	2	0	7
Median female age (years[range])	32 [21-40]	31.5 [31-32]	0 [0-0]	32 [21-40]
Number of male subjects	7	2	0	9
Median male age (years[range])	34 [20-52]	43.5 [20-67]	0 [0-0]	34 [20-67]
Total	213	31	1	245

Supplementary material 2

HPLC rate-blanked pharmacophenotyping method description

The enzymatic reaction takes place in the chromatographic vial placed inside the HPLC autosampler that is set to 37.0 °C. Calibration is performed with every patient specimen using 0.01 mmol/l solution of 6-MMP. The Sarstedt K3EDTA tube specimen is delivered to the laboratory at the earliest possible time, but if needed an intact specimen may be stored refrigerated for up to six days. Upon analysis, the vial is gently swirled using the hematologic agitator and 300 µl of full blood is first taken for SNP testing. The vial is centrifuged at 350 x g, plasma discarded and the remaining blood is filled in the original tube with approx. 3 ml of 0.9% Sodium Chloride Solution, resuspended and centrifuged again at 350 x g – this step is repeated three times. Finally, the hematocrit of the erythrocyte suspension is measured using the SYSMEX 5000XE hematologic analyzer and adjusted to 0.30 using 0.9% Sodium Chloride Solution – this step is necessary as the patient RBC counts may vary widely. The lysate is prepared using 1 volume of RBC suspension to which 4 volumes of cold water are added. The lysate is then centrifuged in a refrigerated centrifuge (350 x g), aliquoted and frozen until analysis.

HPLC rate assay: Prior to analysis, the Waters Alliance system was set to the following conditions: flow rate 0.5 ml/min, column temperature 40 °C and autosampler temperature 37 °C. The vials were incubated inside the autosampler compartment of the chromatograph. The gradient was programmed to allow separation of the reaction product, 6-methylmercaptopurine within 15 min cycle including column Supelco LC18S 150x3 mm equilibration. A sampling cycle was 15 min and aliquots of vials were sampled in a way that the reaction blank vial was injected at 0, 30, 60, 90, 120 and 150 min and the sample vial at 15, 45, 75, 105, 135 and 165 min; the sample blank vial was injected once at 180 min. With each reaction course a pure standard of 6-methylmercaptopurine dissolved in DMSO was analyzed and its nominal peak area used for quantitation of the reaction product formed. For each patient specimen, three reactions are prepared in the “vortexable” eppendorf vials: the sample blank, the reagent blank and the patient specimen whose composition is as follows: sample blank (500 µl of RBC lysate, zero volume of 120 mmol/l 6-MP in DMSO and 50 µl of phosphate buffer), the reagent blank (zero volume of RBC lysate, 50 µl of 6-MP and 500 µl of phosphate buffer, pH = 7.5), the patient specimen (500 µl of RBC lysate, 50 µl of 6-MP and no phosphate buffer). The reaction is then started with a cosubstrate, S-adenosylmethionine (S-AM) in phosphate buffer at the concentration of 0.775 mmol/l. The activated reaction mixture is vortexed, the whole amount transferred into the chromatographic vial and placed in the preheated chromatograph autosampler.

Signal processing and enzyme activity calculation

The signal of the ”sample blank” should be zero and is used to control for the background noise if any. It is sufficient to check the sample blank only once per analytical batch, as we concluded after performing about a thousand chromatographic runs. If nonlinear, then it usually indicates the need for column replacement. The regression line of the ”reagent blank” is subtracted from the regression line of the ”patient sample” thus correcting for the nonspecific methylation of 6-MP and reagent variations. The amount of 6-MMP formed per 60 minutes reflects TPMT activity, expressed in nmol/hour/ml of packed red blood cells.

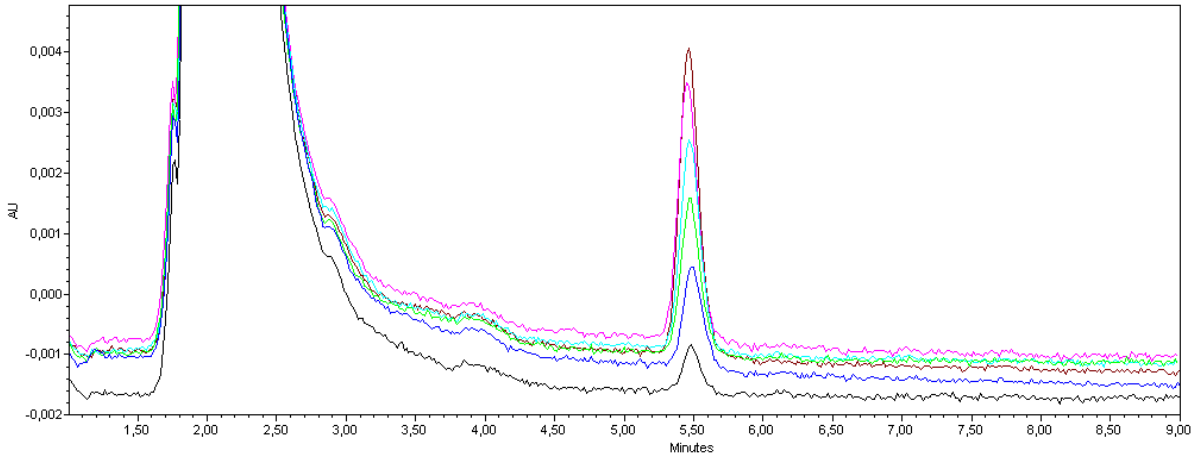

Figure. HPLC chromatograph – monitoring of 6-methylmercaptopurine concentration (retention time 5.5 min) increase in 30 min time increments as the TPMT-catalyzed reaction proceeds.

Supplementary material 3

The interpretive guide for clinical summary on TPMT study. Genotypes are designated as G1-G4, pharmacophenotypes designated as F1-F4.

	G1 (wt -*1/*1)	**G2 (*mut/*1)**	**G3 (*mut/*mut)**	**G4 (*mut#1, *mut#2)**
F1 ↑	*Summary:* Normal genotype, increased TPMT activity			
	Interpretation: Patient is unlikely at increased risk of toxicity after standard dose of thiopurines. Accelerated metabolism of thiopurines may lead to reduced therapeutic effect of standard dosing of thiopurines.			
F2 N	*Summary:* Normal TPMT activity and normal genotype.	*Summary:* Normal TPMT activity, mutation (*enter mut*) heterozygote.		*Summary:* Normal TPMT activity. Detected mutation (*enter mut #1*) and (*enter mut #2*) are likely in haplotype (*enter*) /*1 (wt) heterozygous status.
	Interpretation: Patient is unlikely at increased risk of toxicity after standard dose of thiopurines.	*Interpretation:* Increased toxicity after standard dose of thiopurines cannot be excluded. Careful observation of blood cell count during initiation of thiopurine therapy is recommended.		*Interpretation:* Increased toxicity after standard dose of thiopurines cannot be excluded. Careful observation of blood cell count during initiation of thiopurine therapy is recommended.
F3 ↓	*Summary:* Decreased TPMT activity. Normal genotype (polymorphism in uninvestigated loci cannot be excluded).	*Summary:* Decreased TPMT activity. Mutation (*enter mut*) heterozygote.	*Summary:* Decreased TPMT activity. Mutation (*enter*) homozygote.	*Summary:* Decreased TPMT activity. Detected mutation (*enter mut #1*) and (*enter mut #2*) are likely in haplotype (*enter*) /*1 (wt) heterozygous status.
	Interpretation: Possible risk of increased toxicity after standard dose of thiopurines. Careful	*Interpretation:* Probable risk of increased toxicity after standard dose of thiopurines. Careful	*Interpretation:* High risk of severe toxicity after standard dose of	*Interpretation:* Probable risk of increased toxicity after standard dose of thiopurines. Careful observation of

	observation of blood cell count during initiation of thiopurine therapy is recommended.	observation of blood cell count during initiation of thiopurine therapy is recommended. Dose reduction of thiopurines should be considered.	thiopurines.	blood cell count during initiation of thiopurine therapy is recommended. Dose reduction of thiopurines should be considered.
F4 ↓↓			*Summary:* TPMT activity very low/deficient. Mutation (*enter*) homozygote.	*Summary:* TPMT activity very low/deficient. Detected mutation (*enter mut #1*) and (*enter mut #2*) are likely in compound heterozygous status.
			Interpretation: Very high risk of severe toxicity after standard dose of thiopurines.	*Interpretation:* Very high risk of severe toxicity after standard dose of thiopurines.
Enzyme activity "…" nmol/hour/ml RBC				
F1	*"higher than 24,9"*		*"…" corresponds to*	increased TPMT activity.
F2	*"14,0 – 24,9"*			normal TPMT activity.
F3	*"5,0 – 13,9"*			decreased TPMT activity.
F4	*"lower than 5,0"*			deficient TPMT activity.