TPMT and *NUDT15* Genotyping for Comprehensive Detection of Thiopurine Sensitivity in Multi-Ethnic Populations

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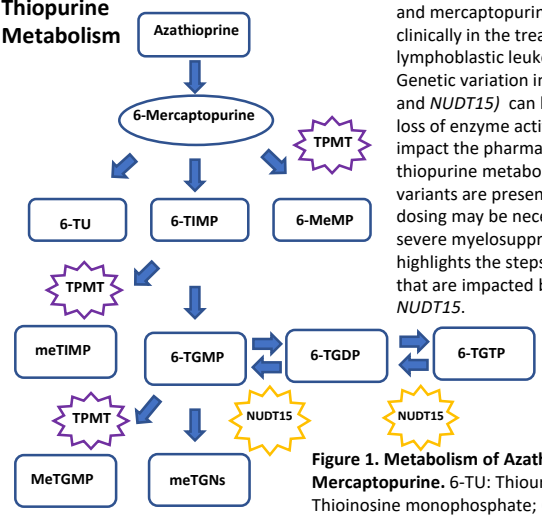
Background

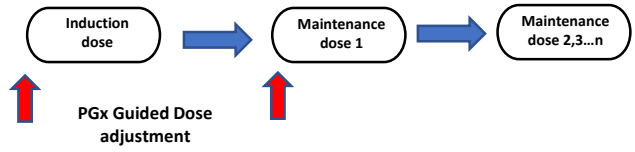
Thiopurine Metabolism



Thiopurines, including azathioprine and mercaptopurine, are used clinically in the treatment of acute lymphoblastic leukemia (ALL)[1]. Genetic variation in two genes (*TPMT* and *NUDT15)* can lead to reduced or loss of enzyme activity, which can impact the pharmacokinetics of thiopurine metabolism. If these variants are present, changes in drug dosing may be necessary to prevent severe myelosuppression[1-3]. **Figure 1** highlights the steps in metabolism that are impacted by *TPMT* and *NUDT15*.

Figure 1. Metabolism of Azathioprine and Mercaptopurine. 6-TU: Thiouric Acid; TIMP: Thioinosine monophosphate; 6-MeMP : 6-methyl mecaptopurine; 6-MeTIMP: 6-Methyl thioinosine monophosphate; 6-TGMP:6-Thioguanine Monophosphate; 6-TGDP:6-thioguanine-diphosphate; 6-TGTP: 6-thioguanine-triphosphate; MeTGMP: methylthioguanosine monophosphate; meTGNs: methyl 6-thioguanosine mono-, di-, or triphosphates.

Thiopurine dosing



NUDT15 SNP frequency is population dependent

Figure 3. Percentage of populations that carry polymorphisms in NUDT15

Genetic variants in TPMT and NUDT15 have been associated with thiopurine related myelotoxicity requiring dose adjustment during treatment of acute lymphoblastic leukemia (ALL). The frequency of variants in these genes varies across different populations[1-3] and comprehensive, accurate genotyping, with a short turnaround time (TAT) is critical to adjust thiopurine dosing. **Figure 3** shows the percent of individuals in a population to carry a currently described NUDT15 polymorphism[1] (NUDT15*1-*9)

Methods and Results

- NUDT15 and TPMT genotyping was performed on genomic DNA (gDNA) acquired from the Coriell Institute (from LCL cell lines; n=20) or isolated from blood and saliva (n=20) in house. Ethnicities included in the study: African, European, Latino, South Asian and East Asian.
- Real time PCR (RT-PCR) and TaqMan™ SNP genotyping was run. Samples were also run for comparison on the PharmacoScan™ (pScan) platform in a CLIA certified diagnostic laboratory. Analysis focused on known PGx relevant haplotypes (TPMT*1, *2, *3A-D, *4, *8, *24 and NUDT15*1-*9.)
- Additionally, on a subset of samples targeted next generation sequencing (NGS) was performed on the Illumina iSeq Sequencing System. All samples (gDNA from blood, saliva or cell line) passed quality control (QC) metrics for all haplotypes interrogated.

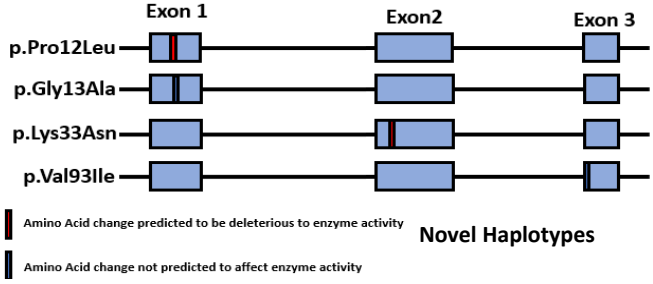
Methods and Results

Sample Type	Concordance with 1000 genomes	Concordance blood and saliva	Concordance with PharmacoScan results*
Blood (n=20)	**N/A**	**100%**	**100%**
Saliva (n=20)	**N/A**	**100%**	**100%**
gDNA	**100%**	**N/A**	**100%**

Table 1. SNP Genotyping results and concordance. *PharmacoScan™ assay only genotypes for NUDT15*3. All of the TPMT variants included in the genotyping panel are also present on pScan.

Sample Type	Coding region covered (TPMT and NUDT15)	Concordance blood and saliva	Concordance with rt-qPCR
Blood	**100%**	**100%**	**100%**
Saliva	**100%**	**100%**	**100%**
gDNA	**100%**	**N/A**	**100%**

Table 2. NGS Performance. Libraries were prepared from gDNA extracted from either blood or saliva (same individual) or acquired from the Coriell Institute. Libraries prepared from all sample types meet the minimum QC requirements for sequencing. Libraries were normalized and pooled and then subsequently sequencing on the Illumina iseq NGS platform. Alignment was performed on instrument and was visualized using Integrative Genomics Viewer (IGV).



Coriell ID	Ethnicity	NUDT15 Amino Acid Change	Minor Allele Frequency	protein modeling Predict impact
NA20845 HG03673	**GUJARATI INDIAN, USA; Sri Lankan Tamil, UK**	**p.Pro12Leu**	**0.01 to 0.20**	**deleterious**
HG02840	**LUHGAMBIA, GAMBIA**	**p.Gly13Ala**	**0.0008-0.08**	**No impact**
NA19403	**LUHYA, KENYA**	**p.Lys33Asn**	**0.003 to 0.08**	**deleterious**
HG02790 HG03019	**PAKISTANI, PAKISTAN**	**pVal93Ile**	**0.02 to 0.31**	**No impact**

Table 3. NGS sample Information.

Conclusions

- Comprehensively genotyping both TPMT and NUDT15 allows for more accurate phenotype prediction of thiopurine sensitivity across multiple ethnic groups.
- Our RT-qPCR assay accurately genotypes for all NUDT15 haplotypes with CPIC recommendations and our NGS assay allows for the detection of novel alleles.

References

1. Relling MV, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes:2018 Update. Clin Pharmacol Ther. 2019 May;105(5):1095-1105.
2. Singh M, et al. Emerging role of NUDT15 polymorphisms in 6-mercaptopurine metabolism and dose related toxicity in acute lymphoblastic leukaemia. Leuk Res. 2017 Nov;62:17-22.
3. Zimdahl KA, et al. Comprehensive study of thiopurine methyltransferase genotype, phenotype, and genotype-phenotype discrepancies in Sweden. Biochem Pharmacol. 2019 Apr 18