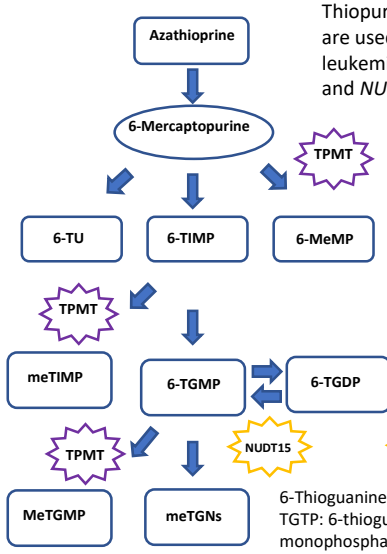
Four Novel NUDT15 Haplotypes Relevant for Treatment of Acute Lymphoblastic Leukemia (ALL)

Amy Turner[1], Praful Aggarwal[1], Gunter Scharer[1], and Ulrich Broeckel[1]

[1]RPRD Diagnostics LLC, Wauwatosa, WI 53226

Introduction



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[1-4]. **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 mercaptopurine; 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.

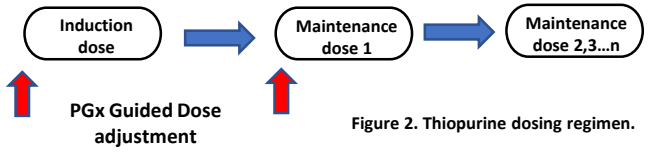


Figure 2. Thiopurine dosing regimen.

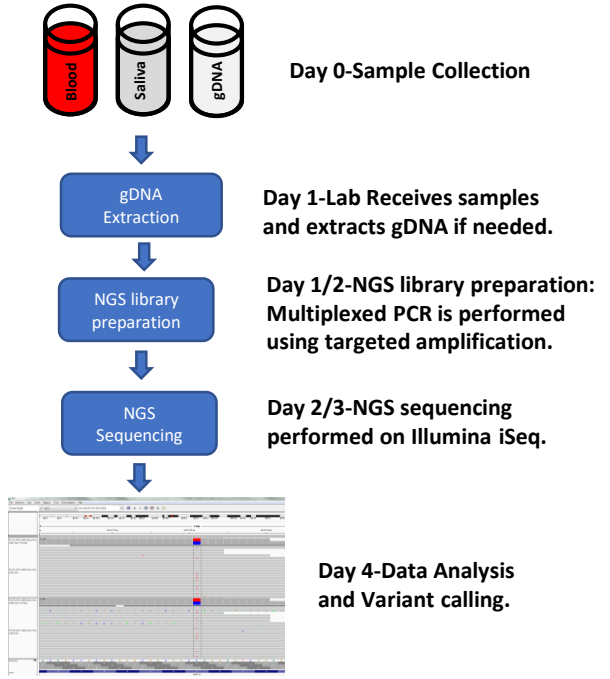
NUDT15 SNP frequency is population dependent

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 (Ref) 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 (Ref) *(NUDT15**1-*9)[1]

Figure 3. Percentage of populations that carry polymorphisms in NUDT15. *Includes Americans of African descent. **Includes Americans of European descent.

Methodology

Figure 4. Testing methodology.



Results

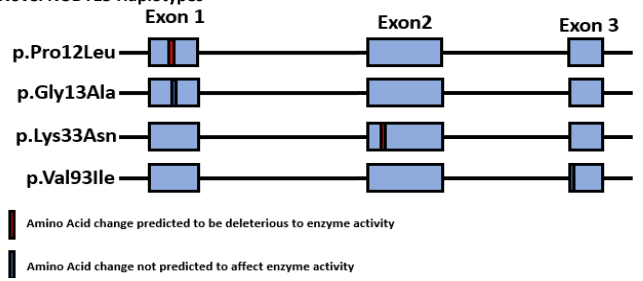
Sample Type	Coding region covered (TPMT and NUDT15)	Concordance blood and saliva	Concordance with rt-qPCR
Blood	100%	100%	100%
Saliva	100%	100%	100%
gDNA	100%	N/A	100%

Table 1. 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)[4].

Results

Figure 5. Novel NUDT15 Haplotypes



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 to enzyme activity
HG02840	LUHGAMBIA, GAMBIA	p.Gly13Ala	0.0008-0.08	No impact to enzyme activity
NA19403	LUHYA, KENYA	p.Lys33Asn	0.003 to 0.08	deleterious to enzyme activity
HG02790; HG03019	PAKISTANI, PAKISTAN	pVal93Ile	0.02 to 0.31	No impact to enzyme activity

Table 2. NGS Performance. Sample Data on was acquired from the 1000 genomes project[5].

Conclusions

In summary, our methodology is able to accurately genotype *TPMT* and *NUDT15* in gDNA samples extracted from blood and saliva or acquired from the Coriell institute. We covered 100% of the known SNPs currently assigned to both *TPMT* and *NUDT15* haplotypes and were also able to characterized four novel *NUDT15* haplotypes. Importantly, our assay allows for detection of both known and novel polymorphisms in all ethnic groups.

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
4. James T. Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S. Lander, Gad Getz, Jill P. Mesirov. Integrative Genomics Viewer. Nature Biotechnology 29, 24–26 (2011).
5 A global reference for human genetic variation, The 1000 Genomes Project Consortium, Nature 526, 68-74 (01 October 2015) doi:10.1038/nature15393.

For our additional NUDT15 and TPMT research: