Abstract P062

Development and validation of an NGS assay for the detection of clinically actionable genetic variants in *DPYD* and *UGT1A1*

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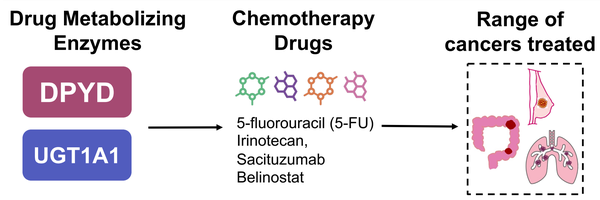
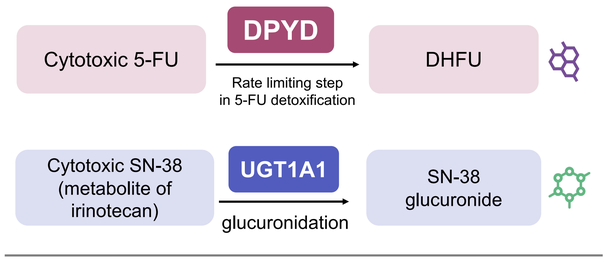
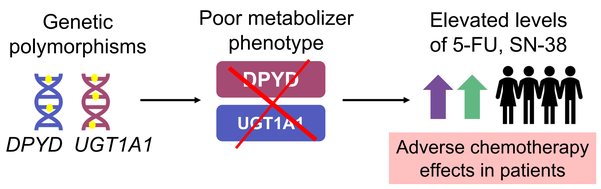
INTRODUCTION

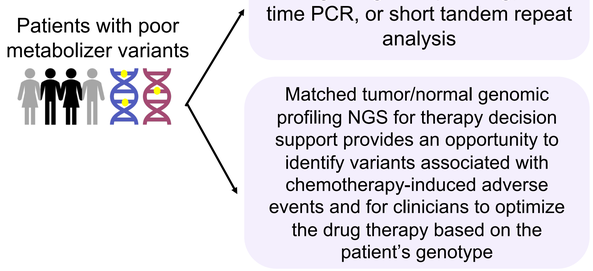


Dihydropyrimidine dehydrogenase (DPYD)

UDP-glucuronosyltransferase isoform 1A1 (UGT1A1)







Here, we report the validation of an NGS assay which includes a novel repeat polymorphism calling algorithm for the detection of *DPYD* and *UGT1A1* genetic variants from Tempus xT, a NGS paired tumor/normal 648-gene assay for cancer therapy decision support.

METHODS

- 7 SNV and 3 TA repeats in *DPYD* (NM_000110.4) and *UGT1A1* (NM_000463.3): ***DPYD *2a, DPYD *13, DPYD HapB3, DPYD c.557A>G, DPYD c.2846A>T, UGT1A1 *6, UGT1A1 *27, UGT1A1 *28, UGT1A1 *36,*** and ***UGT1A1 *37*** were targeted by NGS.
- Variant calling of SNV we used Google's DeepVariant software and for *UGT1A1* repeat diplotypes, we implemented a novel calling algorithm that is resilient to stutter created by DNA polymerase in repeats
- The calls of the TA repeat polymorphisms were orthogonally confirmed by a CLIA/CAP lab using PCR amplified capillary electrophoresis (short tandem repeat polymorphism - STRP) and the SNVs were orthogonally confirmed using Sanger sequencing.
- Positive Percent Agreement (PPA) = 100 x (TP)/(TP+FN), Negative Percent Agreement (NPA) = 100 x (TN)/(TN+FP)

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SUMMARY

Our method allows for clinical ***DPYD*** and ***UGT1A1*** genotyping from NGS data collected for tumor profiling, enabling clinicians to **consider potential adverse drug reactions** (loss or decreased function of DPYD/UGT1A1) **simultaneously with therapy selection** for cancer patients.

RESULTS

Genetic variants of *DPYD* and *UGT1A1* were selected based on their enzyme activity, the strength of clinical evidence, population frequency and toxicity risk of 5-FU and irinotecan (respectively) (Table 1). Subpopulations with > 1% frequency shown in bold

Table 1 - Genetic variants of *DPYD* and *UGT1A1* associated with the adverse events

Gene	Allele	Consequence	Allele Frequency (%)	European (%)	African (%)	East Asian (%)	South Asian (%)	Latino (%)
DPYD	*2A	c.1905+1G>A	0.58	0.58	0.06	0.00	0.43	0.11
DPYD	*13	p.Ile560Ser	0.03	0.06	0.01	0.00	0.00	0.00
DPYD	HapB3	p.Glu412Glu	**1.38**	**2.11**	0.23	0.03	**1.73**	0.45
DPYD	c.557A>G	p.Tyr186Cys	0.21	0.00	**2.15**	0.01	0.00	0.07
DPYD	c.2846A>T	p.Asp949Val	0.28	0.51	0.08	0.01	0.05	0.26
UGT1A1	*6	p.Gly71Arg	**2.15**	0.20	0.07	**15.30**	**1.96**	**2.40**
UGT1A1	*27	p.Pro229Gln	0.14	0.00	0.00	**1.95**	0.03	0.01
UGT1A1	*36	c.-53_-52TA[6]	**2.2**	**7.1**	0.4	0	0.3	0.1
UGT1A1	TA_reference	c.-53_-52TA[7]	**61.0**	**47.1**	**66.7**	**87.8**	**67.2**	**58.5**
UGT1A1	*28	c.-53_-52TA[8]	**34.7**	**40.4**	**32.4**	**12.2**	**32.5**	**41.2**
UGT1A1	*37	c.-53_-52TA[9]	1.6	**5.3**	0.5	0	0.1	0.2

A total of 199 unique samples consisting of DNA extracted from characterized genotypes in the reference standards (GetRM cell line repository and Coriell cell lines), clinical saliva specimens or clinical blood specimens as detailed in Table 2. Samples were sequenced on the Illumina NovaSeq 6000 using the Tempus xT.v4 assay. Samples were tested at different DNA inputs (Table 3). TA calling algorithm requires a minimum depth of 70x for 100% accuracy and this coverage was exceeded this in all samples. All gene specific positions in reference standards and positive positions in clinical samples were confirmed

Table 2: Samples utilized for validation and the variant types

Variant Type Validated	Validation Samples	Blood Specimens	Saliva Specimens	Orthogonal validation
DPYD SNV	56 clinical samples 21 clinical reference	67	10	Sanger
UGT1A1 SNV	43 clinical samples 45 reference standards	31	12	Sanger
UGT1A1 TA Repeat	53 clinical samples 50 reference standards	41	12	STRP

Table 3: The NGS performance SNV and indel calling

Target	Value (mean)
DNA quantity	25-600 ng
SNV read depth	DPYD: minimum = 138 (935) UGT1A1: minimum = 688 (2314)
Indel read depth	minimum = 92 (723)

Accuracy of *DPYD* assay was evaluated using 21 samples with verified genotype (14 positive and 7 negative samples) from an early version of the Tempus xT assay (CLIA/CAP lab test, xT.v2). Additionally, accuracy of *UGT1A1* was established with 50 (43# positive and 12 negative) total specimens selected from the GeT-RM repository or from the Coriell database (PMID: 26621101, see external references) based on their reported diplotype. A combination of heterozygous and homozygous alleles at the clinical sites were tested. The Negative (*1) targeted allele is considered negative for this analysis and samples were evaluated at all gene-specific targeted positions. 100% NPA and PPA for both SNP and indel alleles was observed.

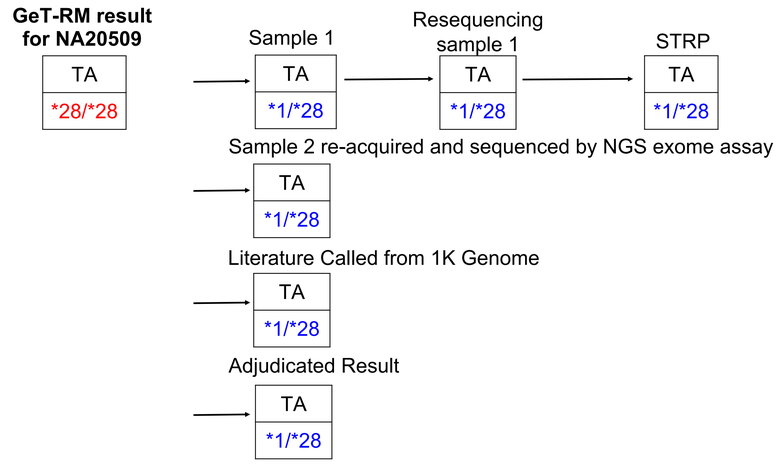
Table 4: Accuracy of *DPYD* and *UGT1A1* to reference samples

Gene	Clinical Target	Total Samples	# Heterozygous samples	# Homozygous samples	PPA (%)	NPA (%)
DPYD	c.557A>G	3	3	0	100	100
DPYD	*2A	4	4	0	100	100
DPYD	*13	3	3	0	100	100
DPYD	HapB3	2	2	0	100	100
DPYD	c.2846A>T	2	2	0	100	100
DPYD	Negative (*1)	7	0	7	100	100
UGT1A1	*36	7#	7	0	100	100
UGT1A1	*37	5#	5	0	100	100
UGT1A1	*28	26#	21++	5	100	100
UGT1A1	*27	1#	1	0	100	100
UGT1A1	*6	4	3	1	100	100
UGT1A1	Negative (*1)	12	0	12	100	100

#Subset of samples were counted more than once.
++ GeT-RM sample NA20509 is referenced as homozygous *28, however after confirmation via STRP we determined it to be heterozygous *28 and included in table as *28 het (See Figure 1 for details). Target allele count is evaluated if the allele and its genotype was previously identified in the reference set and was confirmed in assay.

The discordant sample was sequenced twice with targeted panel and sent for orthogonal testing (Figure 1). Sample was repurchased and genotyped by a Tempus exome panel and was found discordant. Stargazer, a recently published haplotypecaller for drug metabolizing genes used public WGS BAM files available for the 1000 Genomes Project samples and called this position *1/*28. In all scenarios the samples were discordant and showed heterozygous *28 instead of GeT-RM published homozygous *28.

Figure 1 – Discordant Investigation



To establish the sensitivity and specificity of the *DPYD* and *UGT1A1* assays, the genotype results of the validation samples (56 and 72 respectively) were compared to the results of orthogonal testing (Table 5). The positive samples were orthogonally tested at the locus of interest and confirming it as positive (Hetero 0/1,1/2,1/1). The negative samples (Homo 0/0) were sequenced at all sites of clinical interest to confirm reference genotype and their negative status. We observed 100% concordance between the targeted alleles and sanger confirmation or STRP done at the reference lab. This gives an analytical sensitivity and specificity of 100% with the targeted SNV and Genotypes.

Table 5: Analytical Sensitivity and Specificity of *DPYD* and *UGT1A1* to Clinical samples

Gene	Alteration	Count of verified clinical samples by zygosity				Sensitivity	Specificity
		Homo (0/0)	Hetero (0/1)	Hetero (1/2)	Homo (1/1)		
DPYD	HapB3	7	12	0	2	100%	100%
DPYD	*13	7	8	0	0	100%	100%
DPYD	*2A	7	7	0	0	100%	100%
DPYD	c.2846A>T	7	9	0	0	100%	100%
DPYD	c.557A>G	7	9	0	2	100%	100%
UGT1A1	*36	23	4	6	1	100%	100%
UGT1A1	*37	23	4	4	0	100%	100%
UGT1A1	*28	23	5	10	5	100%	100%
UGT1A1	*27	23	7	0	1	100%	100%
UGT1A1	*6	23	9	0	6	100%	100%

Insights

- In total 199 samples were evaluated, 128 clinical samples (98 positive, 30 reference controls) were evaluated with 100% accuracy.
- A novel calling algorithm was developed and used for the TA repeat in *UGT1A1* that is resilient to stutter created by DNA polymerase in repeats.
- A discordant result was identified from a GeT-RM reference sample with a homozygous call at the UGT1A1 *28 locus, follow up supported the *UGT1A*1 assay and was confirmed as a heterozygous *28.
- The discordance is potentially due to limitations in the array platform used to initially genotype a complex region in the GetRM sample.

References

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