Extracting pharmacogenetic results from molecular tumor board DNA sequencing and returning CYP2C19 results to cardiology patients considering clopidogrel therapy at Indiana University

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CPIC call, October 3, 2024

Cancer patients are frequently prescribed PGx medications

469 patients from Precision Genomics Clinic

282 (60%) were prescribed ≥1 PGx medication

67 (14%) were prescribed ≥1 PGx medication with actionable genotype

Shugg, et al., 2022, JCO Precis Oncol, Feb;6:e2100312

Validation of Aldy to make PGx calls from WES

- Our molecular tumor board does germline sequencing, but does not report PGx results
- Compared Aldy extracted PGx results from clinical WES to results from IU PGx Lab
- CYP2B6 (2), CYP2C8 (3), CYP2C9 (6), CYP2C19 (7), CYP2D6 (12), CYP3A4 (2), CYP3A5 (3), CYP4F2 (1), DPYD (3), G6PD (2), NUDT15 (2), SLCO1B1 (1), and TPMT (3)
- Confirmed 100% concordance for 59 clinically actionable variants
  - If read depth was >30x for the specific variant
- Cannot do CYP2D6 copy number variations from WES

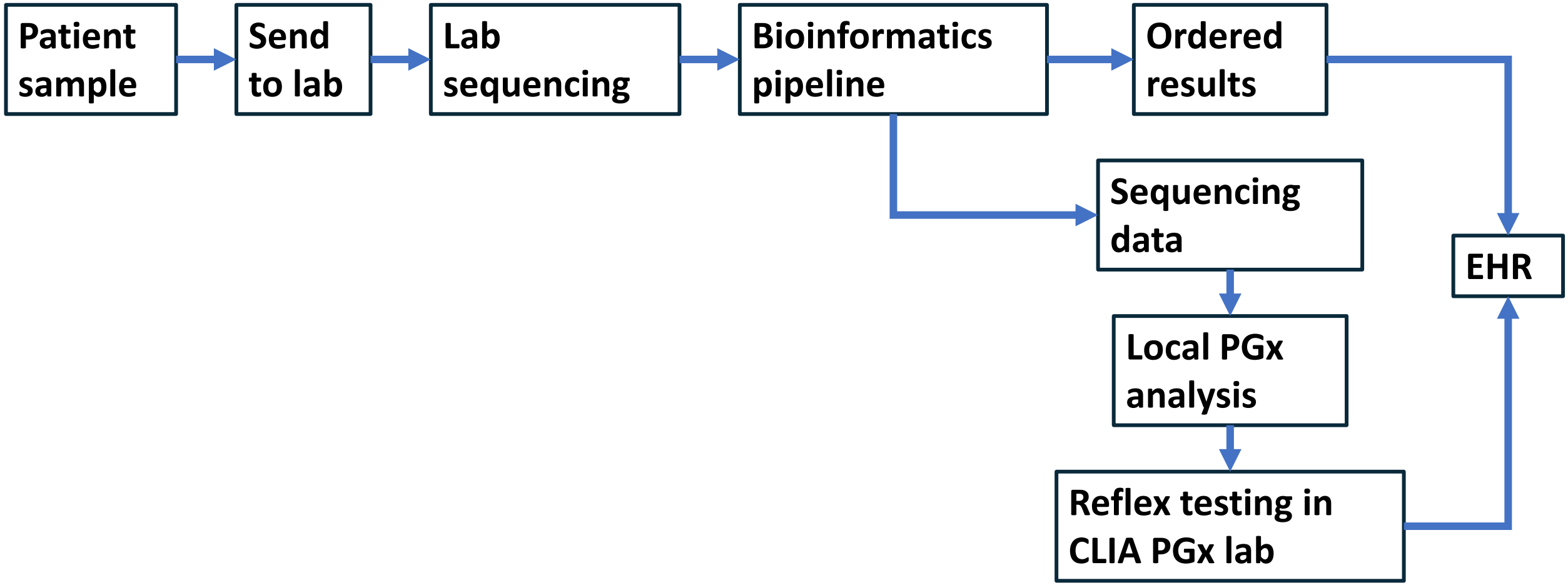
Ly, et al., J Mol Diag 2022 Jun;24(6):576-585

Additional clinically actionable alleles are detected by WES

Gene	Variant alleles	Functional Effect*	Variant Allele Count in Development and Validation Cohorts (Total Chromosomes)	Minor Allele Frequency+		
				African	American Admixed	European
CYP2B6	*8, *13	No Function	2 (328)	<0.001	--	0.004
CYP2C19	*24	No Function	1 (328)	<0.001	--	<0.001
	*35	No Function	2 (328)	0.016	--	<0.001
CYP2D6	*15	No Function	2 (282)	<0.001	0.002	<0.001
	*59	Decreased	3 (282)	0.002	--	0.005
	*62	No Function	1 (282)	<0.001	--	<0.001
DPYD	HapB3	Decreased	2 (226)	0.003	--	0.020
G6PD	A-	Deficient	1 (328)	0.001	--	<0.001
NUDT15	*2, *9	No Function	2 (164)	<0.001	--	0.001

Ly, et al., J Mol Diag 2022 Jun;24(6):576-585

Implementation strategy for repurposing clinical sequencing results to call pharmacogenetic variants



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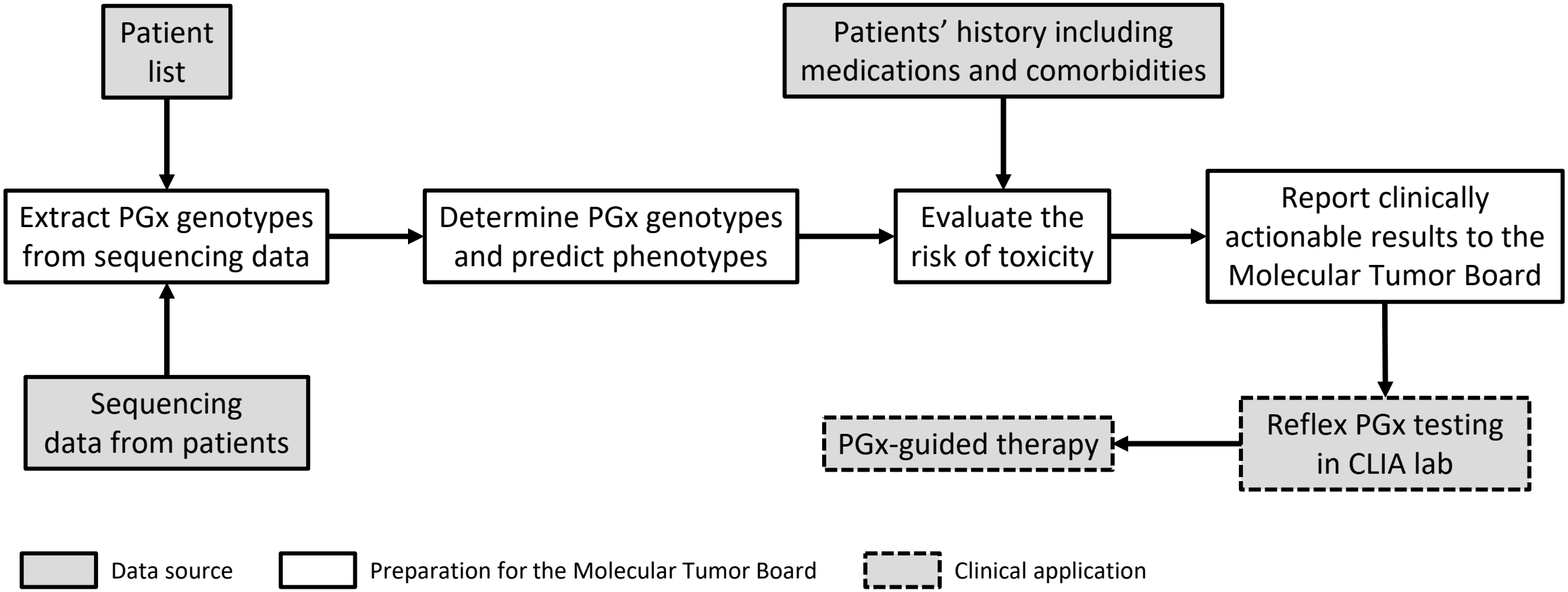
Clinical workflow for PGx analysis of DNA sequencing from molecular tumor board



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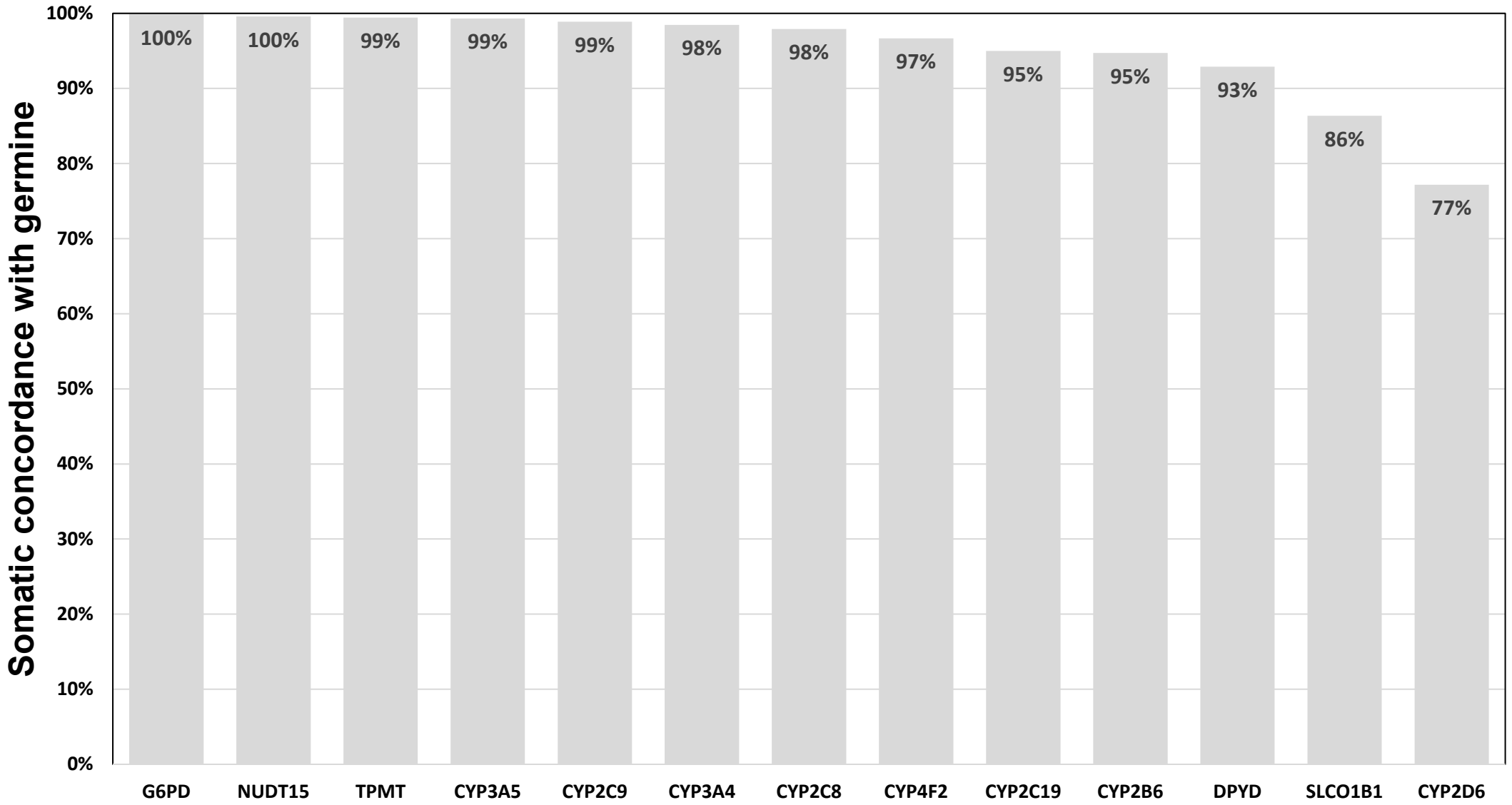
Analysis of the costs and effort for the PGx extraction

- During Jan-Dec 2023, averaged 20.6 patients per week
- Total time per week (after sequencing data received), ~2 days
- Total hands-on time, ~3.25 hrs
  - Computation time to get patient info and match to BAM files ~ 1 hr
  - Downloading BAM files into the active computation environment ~ 2 hrs
  - Aldy run time ~0.75 hrs
  - Assessment of the actionability of the results for individual patients ~0.5 hrs
- Tumor board usually lasts ~ 1 hour
- Using average pharmacist salary + benefits, costs ~$15 per patient
  - Not including reflex testing in clinical PGx testing lab
- Working on an automated process as BAM files are ingested from the sequencing vendor into the LifeOmic Precision Health Cloud

Tumor DNA is another source of sequencing data

- May be WGS, WES, or actionable hot spots
- Tumors include germline DNA from adjacent tissue
- Requires adjustments to bioinformatic parameters
- Complicated by somatic mutations
  - Structural variations
  - Point mutations
  - Chromosomal gains/losses

Squencing from tumors largely reflects germline



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Number of genes =13

Primary Cancer Type	# in cohort	Number of discordances in any genes	Percent discordances
Colorectal	89	45	3.9
Breast	86	59	5.3
Non-small cell lung, Adeno	56	51	7.0
Pancreas	48	17	2.7
Prostate	44	28	4.9
Soft tissue sarcoma	34	22	5.0
Glioblastoma multiforme	29	27	7.2
Head and Neck	28	8	2.2
Cholangiocarcinoma	25	8	2.5
Esophageal	25	20	6.2
Renal	25	15	4.6
Bladder/urothelial	23	18	6.0
Ovarian	18	17	7.3
Melanoma	16	12	5.8

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Gene	rsID	Data type	Median	25th	75th
CYP2D6*2	rs1135840	Germline	231	195	266
		Somatic	391	296	491
CYP2D6*2	rs16947	Germline	412	352	412
		Somatic	573	440	741
CYP2D6*3	rs35742686	Germline	538	460	626
		Somatic	845	633	1075
CYP2D6*4	rs3892097	Germline	734	615	855
		Somatic	1168	882	1517
CYP2D6*6	rs5030655	Germline	578	482	683
		Somatic	940	716	1215
CYP2D6*7	rs5030867	Germline	674	565	766
		Somatic	1070	840	1349
CYP2D6*10	rs1065852	Germline	252	207	301
		Somatic	467	347	582
CYP2D6*14	rs5030865	Germline	909	777	1064
		Somatic	1554	1176	1951
CYP2D6*17	rs28371706	Germline	325	271	379
		Somatic	659	496	842
CYP2D6*41	rs28371725	Germline	817	700	950
		Somatic	1385	1092	1749
CYP3A4*2	rs55785340	Germline	236	209	275
		Somatic	377	276	483
CYP3A4*22	rs35599367	Germline	37	29	45
		Somatic	38	22	57
CYP3A5*3	rs776746	Germline	121	105	138
		Somatic	250	178	319
CYP3A5*6	rs10264272	Germline	242	211	275
		Somatic	435	327	542
CYP4F2*3	rs2108622	Germline	457	400	522
		Somatic	843	646	1018
DPYD*4	rs1801158	Germline	540	483	605
		Somatic	938	698	1240
DPYD*13	rs55886062	Germline	229	202	260
		Somatic	418	309	543
TPMT*2	rs1800462	Germline	360	319	408
		Somatic	635	481	786
TPMT*3B	rs1800460	Germline	166	144	192
		Somatic	363	245	450

The discordant calls from the somatic DNA sequencing frequently do not change the predicted phenotype

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Studying rare toxicities can still identify important pharmacogenetic variants

case reports

Severe Capecitabine Toxicity Associated With a Rare *DPYD* Variant Identified Through Whole-Genome Sequencing

Reynold C. Ly, PhD[1]; Remington E. Schmidt, BS[2]; Patrick J. Kiel, PharmD[1]; Victoria M. Pratt, PhD[3]; Bryan P. Schneider, MD[4]; Milan Radovich, PhD[4]; Steven M. Offer, PhD[2]; Robert B. Diasio, MD[2]; and Todd C. Skaar, PhD[1]

Ly et al., JCO Precis Oncol 2020; PMID: 32923881

DPYD R235Q has impaired enzymatic activity, in vitro

Tested for *in vitro* DPD activity by Steven Offer Lab

Allele	DPD activity	p-value
Wild-type	100%	ref
*2A	0	1E-11
R235W	11%	8E-11
R235Q	14%	5E-9

Thus, we propose the R235Q variant to be actionable, similar to the R235W.

Ly, et al., JCO Prec Oncol 2020

Studying rare toxicities can still identify important pharmacogenetic variants

Life-Threatening Docetaxel Toxicity in a Patient With Reduced-Function CYP3A Variants: A Case Report

Nicholas R. Powell[1], Tyler Shugg[1], Reynold C. Ly[1], Costantine Albany[2], Milan Radovich[2], Bryan P. Schneider[2] and Todd C. Skaar[1]*

Powell et al., Front Oncol 2022; PMID: 35174070

CASE REPORT

- A 58-year-old female with metastatic renal cell carcinoma experienced severe cardiomyopathy (maximum left ventricular ejection fraction drop of 34%) during sunitinib and later axitinib therapy that was reversed upon drug discontinuation.

- Whole genome sequencing data revealed that she was heterozygous for an extremely rare *CYP3A4* variant (rs1483230173; p.P135L) that has not been functionally characterized or curated by PharmVar.

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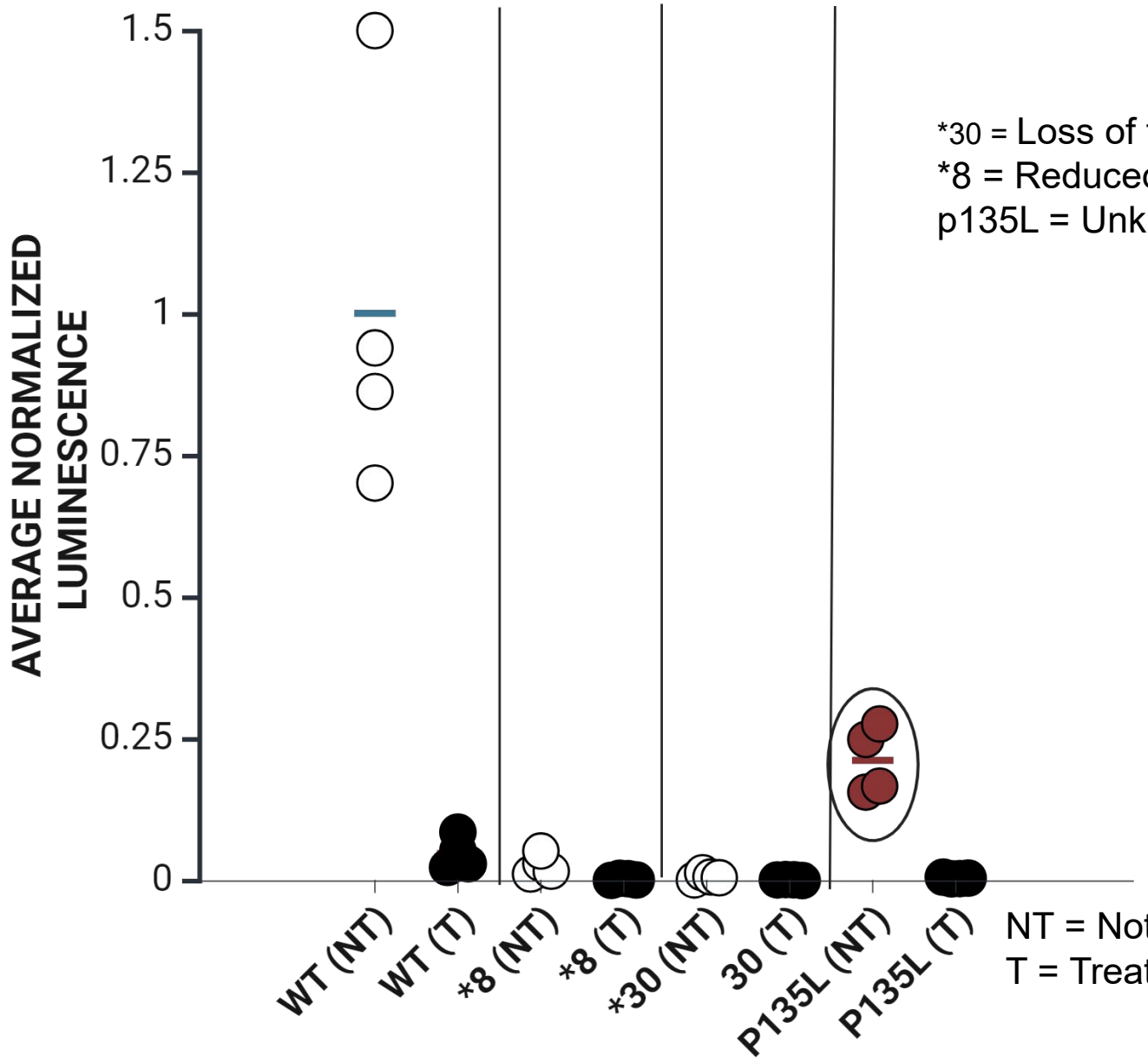

*30 = Loss of function control
*8 = Reduced function control
p135L = Unknown function

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NT = Not treated with Itraconazole
T = Treated with Itraconazole

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Conclusions

1. Whole exome sequencing can provide highly accurate results for many pharmacogenetic genes.
2. Some genes (e.g. *CYP2D6*) require additional input (i.e. copy number results).
3. Tumor DNA can provide important information, but comprehensive genotyping needs to be confirmed with testing from normal DNA sources.

Acknowledgements

Skaar Laboratory
Reynold Ly
Wilberforce Osei
Tayler Smith
Yemi Gafari

Collaborators
Bryan Schneider
Milan Radovich
Steve Bray
Ryan Ratcliff
Ben Salisbury
Vicky Pratt
Amy Breman
Janelle Hodges
Ibrahim Numanagic
Cenk Sahinalp
Peter Schwartz
Tom Doyle
Tyler Shugg
Nick Powell
Steven Offer
Robert Diasio
Patrick Kiel
Emma Tillman
Tom Callaghan
Michael Eadon
Zeruesenay Desta

Funding
NIH (NIGMS, NHGRI)
Indiana University Precision Health Initiative
Indiana University Comprehensive Cancer Center
Indiana Institute for Personalized Medicine
Vera Bradley Breast Cancer Foundation
Zerbe Chair in Pharmacoeconomics