Atrium Health Levine Cancer

Atrium Health Wake Forest Baptist Comprehensive Cancer Center

Real-world impact of an in-house dihydropyrimidine dehydrogenase (*DPYD*) genotype test on fluoropyrimidine dosing, toxicities, and hospitalizations at a multisite cancer center

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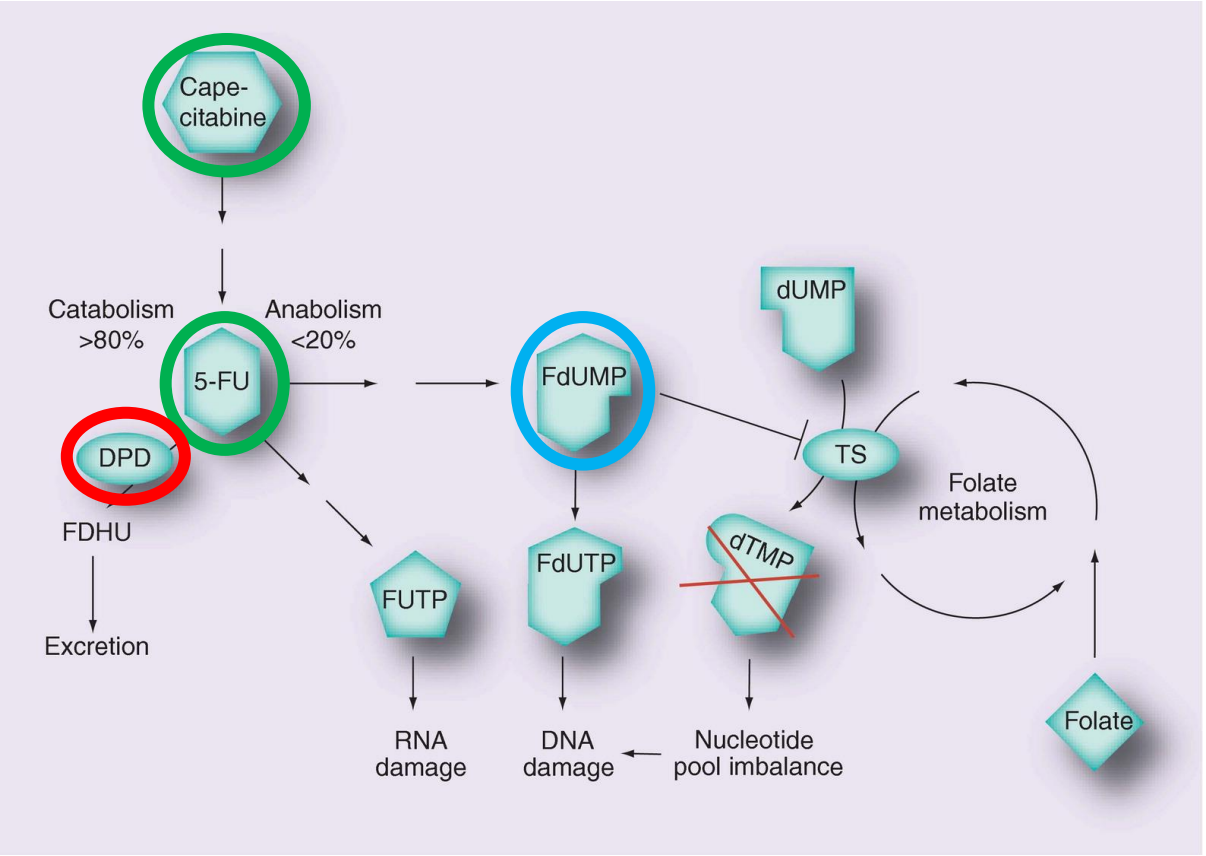
Fluoropyrimidines (FP) & DPD/*DPYD*

- IV 5-fluorouracil (5-FU) and oral prodrug capecitabine (Xeloda)
  - Breast, colorectal, pancreatic, esophageal, head and neck cancers
  - Toxicities: neutropenia, GI, mucositis, and hand-foot syndrome (grade 3/4 ~ 30-35%)
  - Mortality rate from FP-related toxicities: ~0.1-0.5%
- Fluoropyrimidine pharmacology
  - 5-FU bioactivated to FdUMP for efficacy
  - 5-FU exposure determines toxicity
  - 5-FU catabolized by dihydropyrimidine dehydrogenase (DPD/*DPYD*)
    - ~80% of dose metabolized by DPD



Amstutz U, et al. *Clin Pharmacol Ther*. 2018;103(2):210-216.
Slide courtesy of Hertz DL

DPYD Variants with Reduced DPD Activity

Activity	* Allele	rsID	Aliases	Genetic effect	Estimated reduction in enzyme activity	Allele Freq. (Eur)
No function	*2A	rs3918290	c.1905+1G>A, IVS14+1G>A	Splice site	50%	0.8%
	*13	rs55886062	c.1679T>G, p.I560S	Missense	40-50%	0.06%
	N/A	rs67376798	c.2846A>T, p.D949V	Missense	30%	0.4%
Reduced function	N/A	rs56038477	c.1129-5923C>G, HapB3	Nonfunctional transcript	25-30%	2-3%
	N/A	rs115232898	c.557A>G, p.Y186C	Missense	40-45%	1-2% (AA)

- Combined carrier frequency ~5-7% (~1/500 patients homozygous)
- Many other rare/singleton diminished activity variants reported

Amstutz U, et al. *Clin Pharmacol Ther*. 2018;103(2):210-216.

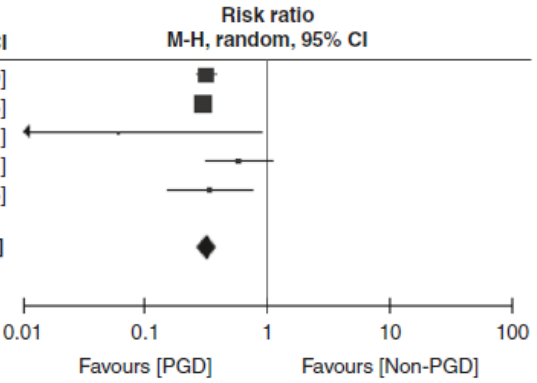
Toxicity and Treatment Outcomes with *DPYD*-guided Dosing Compared to Standard of Care BSA-based Dosing of Fluoropyrimidine

- A systematic review and meta-analysis of 17 eligible studies
  - Sample sizes ranging from 107-2617
  - Inconsistency in *DPYD* testing methods and allele coverage
  - Inconsistency in genotype-guided dose reductions (ranging from 9-83%)
- Reduced incidence of grade 3/4 overall toxicity (RR 0.32, 95% CI 0.27-0.39, p<0.00001)
- No differences for overall response rates (RR 1.49, 95% CI 0.93-1.85, p=0.12)

Grade 3/4 overall toxicity

Study or subgroup	PGD Events	PGD Total	Non-PGD Events	Non-PGD Total	Weight	Risk ratio M-H, random, 95% CI
Deenen, 2016	378	1631	35	48	38.9%	0.32 [0.26, 0.39]
Henricks, 2019	379	1646	66	86	48.4%	0.30 [0.26, 0.35]
Jolivet, 2020	0	8	5	5	0.4%	0.06 [0.00, 0.91]
Lunenburg, 2018	110	793	8	34	7.3%	0.59 [0.31, 1.11]
van Kuilenburg, 2012	4	13	7	7	4.9%	0.34 [0.16, 0.75]
Total (95% CI)		**4091**		**180**	**100.0%**	**0.32 [0.27, 0.39]**
Total events	871		121			

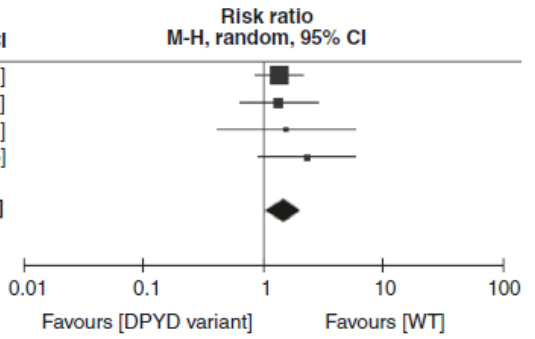
Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 5.84$, df = 4 ($P = 0.21$); $I^2 = 32\%$
Test for overall effect: $Z = 12.32$ ($P < 0.00001$)



Overall response

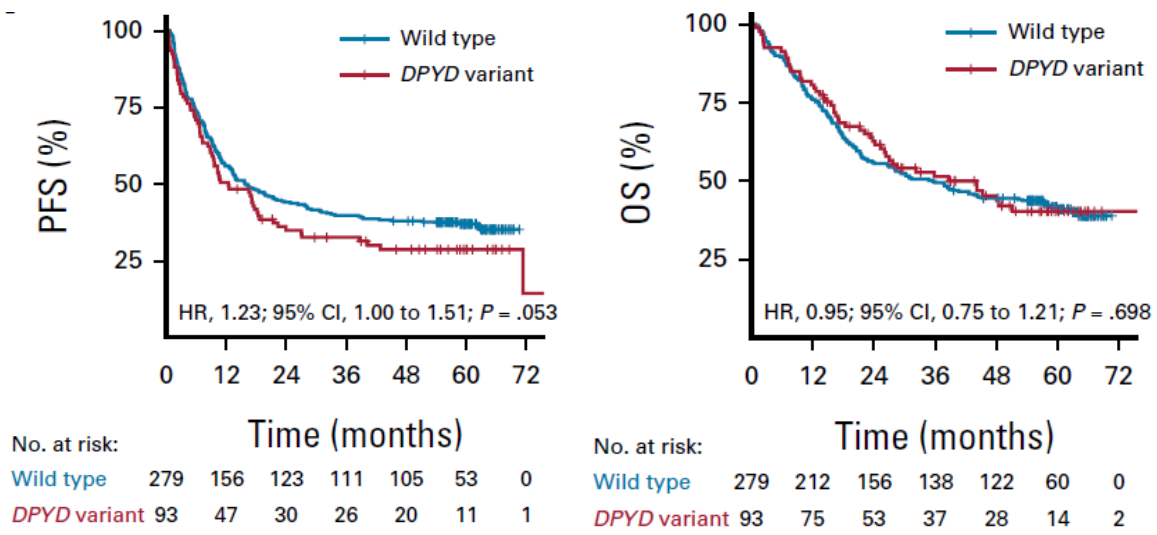
Study or subgroup	DPYD variant allele Events	DPYD variant allele Total	WT Events	WT Total	Weight	Risk ratio M-H, random, 95% CI
Henricks, 2018	16	85	140	1018	56.4%	1.37 [0.86, 2.18]
Henricks, 2019	6	40	179	1606	21.9%	1.35 [0.64, 2.85]
Kleinjan, 2019	2	11	20	174	7.1%	1.58 [0.42, 5.92]
Lunenburg, 2018	4	22	60	771	14.6%	2.34 [0.93, 5.86]
Total (95% CI)		**158**		**3569**	**100.0%**	**1.49 [1.05, 2.12]**
Total events	28		399			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.13$, df = 3 ($P = 0.77$); $I^2 = 0\%$
Test for overall effect: $Z = 2.22$ ($P = 0.03$)

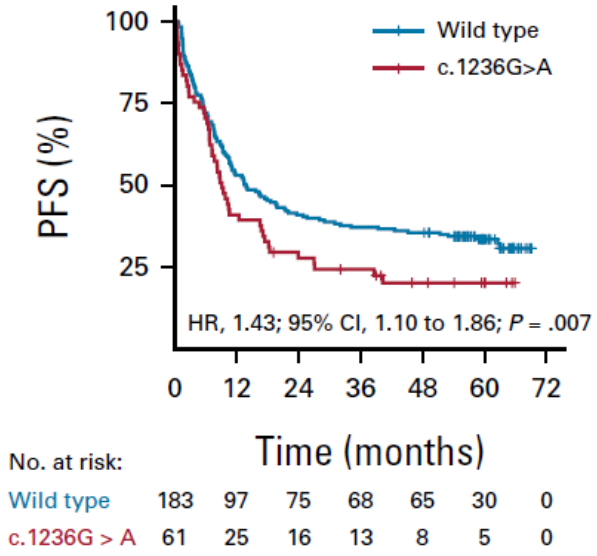


Glewis S, et al. *Br J Cancer.* 2022;127(1):126-136.

Survival Outcomes of *DPYD* Variant Carriers Receiving Genotype-guided Fluoropyrimidine Dosing



Kaplan-Meier plots and HRs for PFS and OS of pooled *DPYD* variant carriers



PFS of pooled *DPYD* c.1236G>A carriers

Knikman JE, et al. *J Clin Oncol.* 2023;41(35):5411-5421.

Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for Fluoropyrimidine and *DPYD*

DPD Phenotype	*DPYD* Diplotype	DPD Enzyme Activity	Dosing Recommendation
Normal metabolizer	Two normal function alleles	Normal	Use label recommended dosage and administration.
Intermediate metabolizer	One normal and one reduced or no function allele, or two reduced function alleles	Decreased	Reduce starting dose by 50% followed by titration based on tolerability
Poor Metabolizer	Two no function alleles, or one no function and one reduced function allele	None	Avoid use or reduce starting dose by ≥ 75% followed by titration based on tolerability

Amstutz U, et al. *Clin Pharmacol Ther*. 2018;103(2):210-216.

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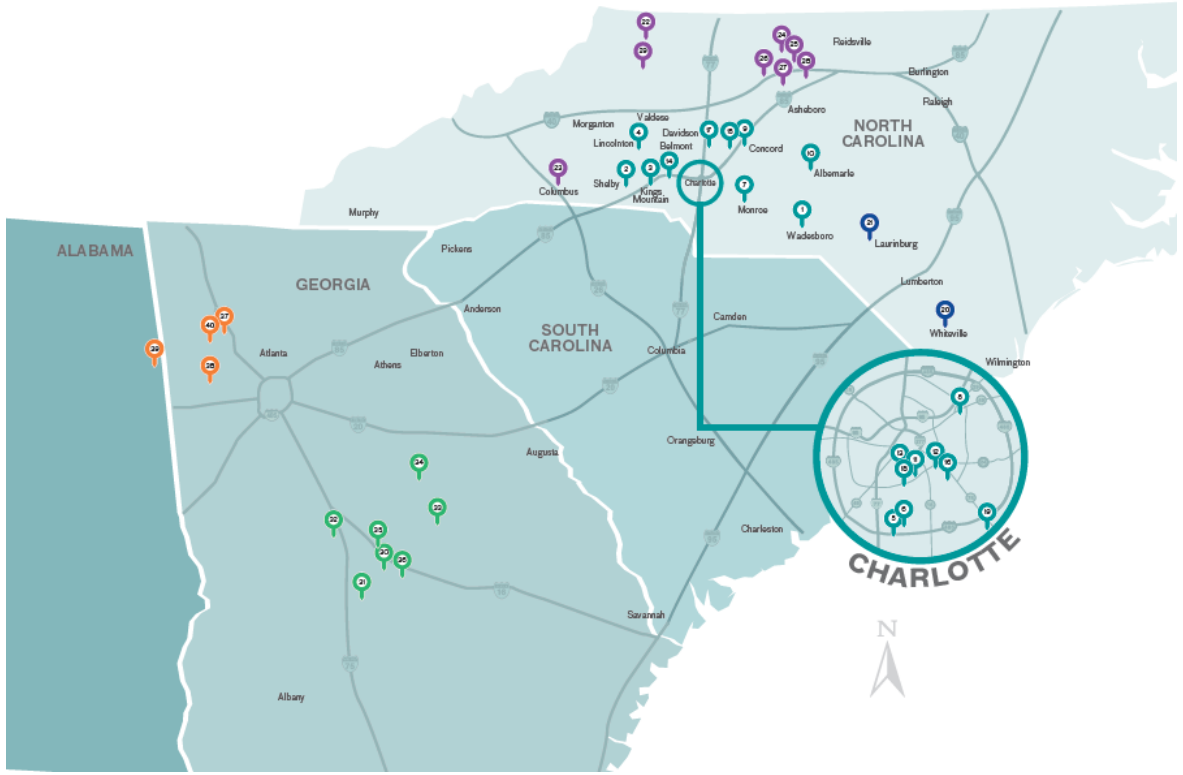
Clinical Implementation of *DPYD* Genotyping Test at Atrium Health Levine Cancer

Atrium Health Levine Cancer



DPYD Testing Workflow

What?

DPYD genotyping

- 5 clinically actionable variants: DPYD*2A(c.1905+1G>A), c.2846A>T, c.1679T>G (DPYD*13), c.1236G>A (haplotype B3), c.557A>G

Where?

LCI Molecular Biology and Genomics Laboratory

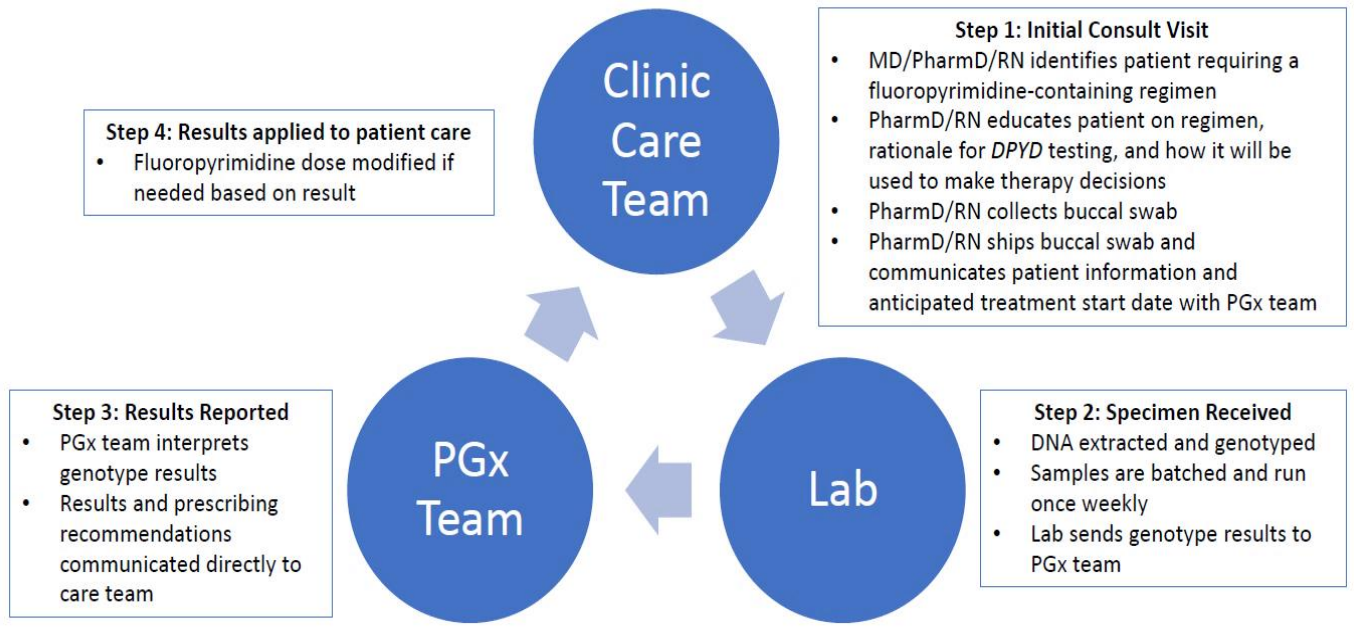
- Clinical Laboratory Improvement Amendment (CLIA)-approved laboratory developed test
- Buccal swab kits available at 1 site (2020) → 2 sites (2021) → 15 sites (2022)
- Option for at-home collection

When?

Results available within 2-7 days

- Batch run twice weekly

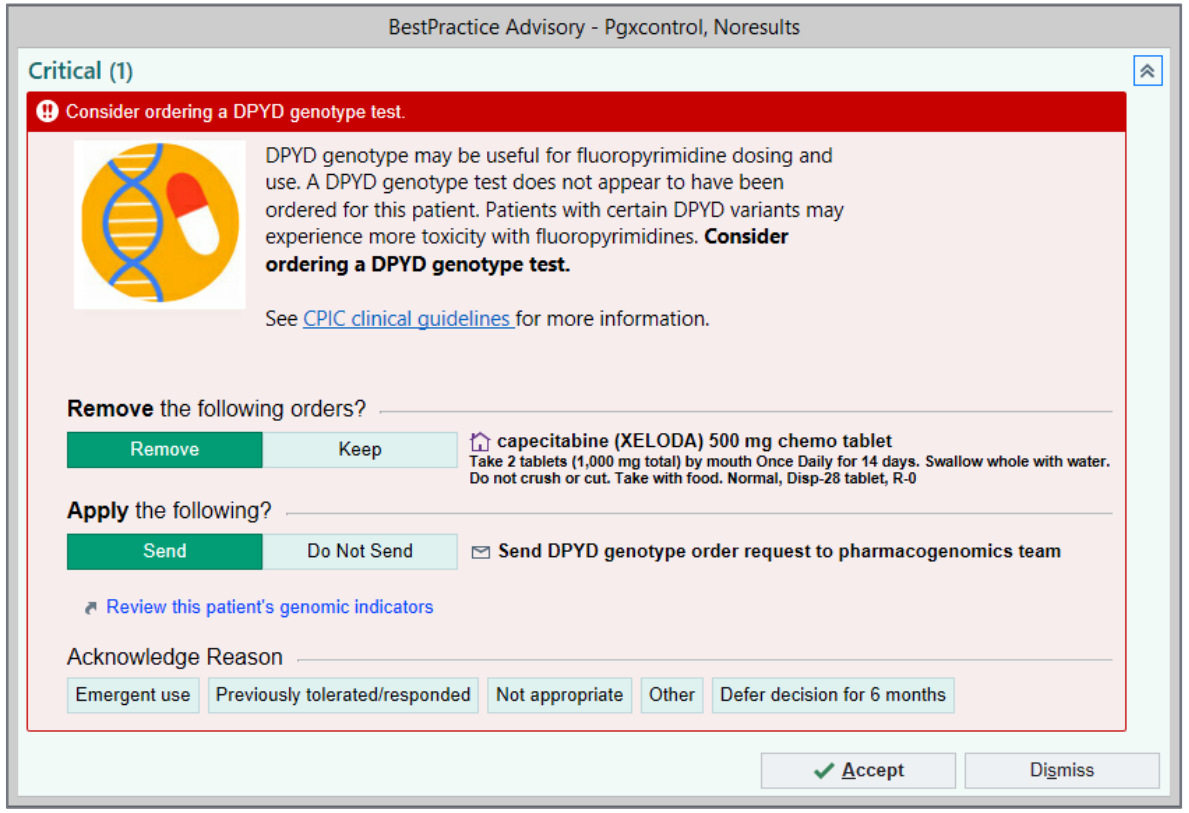
How?



Morris SA…Patel JN. *Am J Health Syst Pharm*. 2023;80(19):1342-1349.

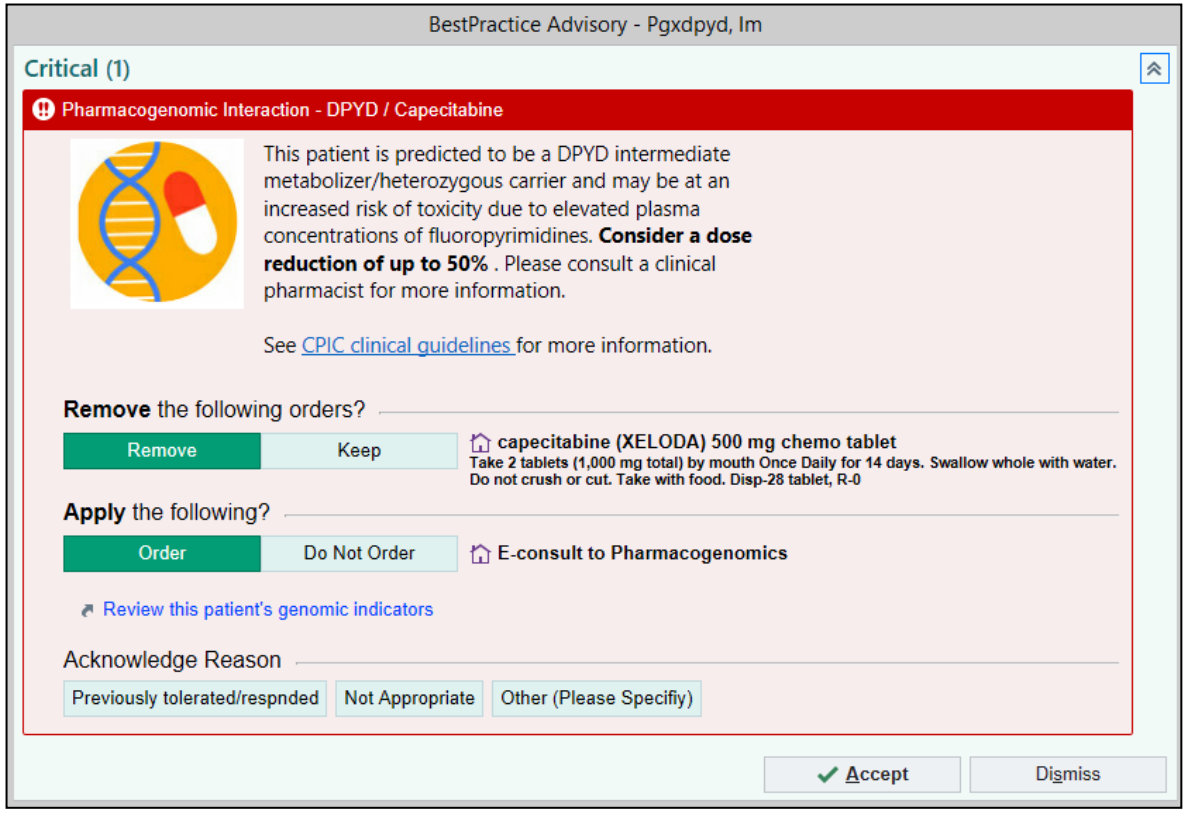
Partly funded by the Heineman Foundation, Inc.

Best Practice Advisory Alerts



Pre-test alert

For patients who have not been tested:
Notifies prescriber to consider ordering a *DPYD* genotype test



Post-test alert

For patients with an observed variant:
Notifies prescriber to consider dose reduction

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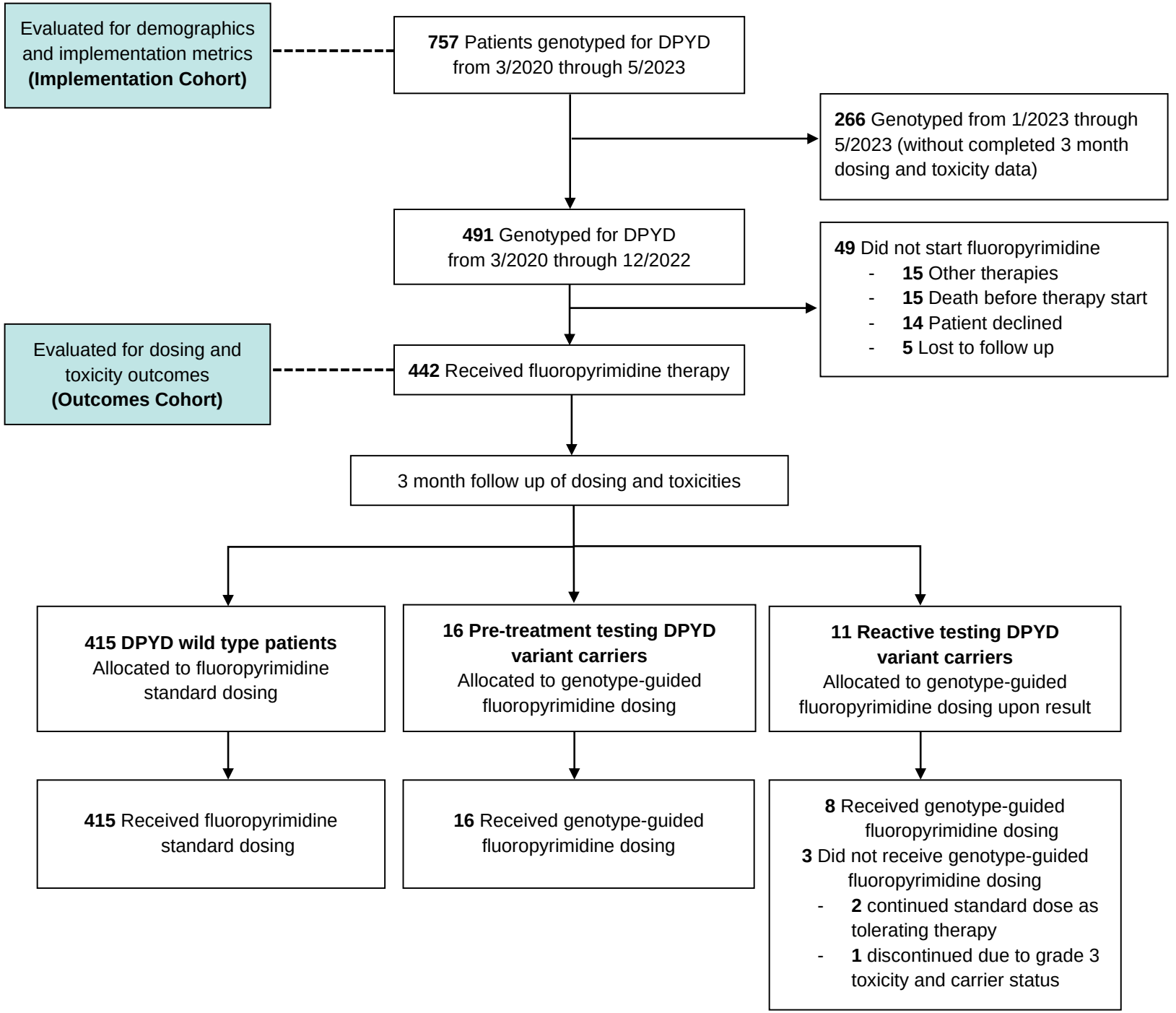
Real-world Outcomes Study

Methods

- Prospective, real-world, observational cohort study across 14 oncology clinics
- Eligible patients: those receiving or planning to receive fluoropyrimidine-based chemotherapy and undergoing in-house *DPYD* genotyping
- Primary endpoint:
  - Proportion of *DPYD* variant carriers receiving genotype-guided treatment modifications
- Secondary endpoints:
  - Mean first cycle relative dose intensity
  - Rates of fluoropyrimidine-related grade 3+ toxicities, hospitalizations, and treatment discontinuations
  - Compared between three groups:
    - *DPYD* wild-type patients
    - Pre-treatment testing *DPYD* variant carriers
    - Reactive testing *DPYD* variant carriers

CONSORT Diagram

- Pre-treatment testing: specimen collected prior to treatment start date
- Reactive testing: specimen collection on or after treatment start date.



Not yet published – DO NOT DISTRIBUTE

Characteristics	All patients (N=757)	DPYD wild type (N=712)	Pre-treatment testing carriers (N=32)	Reactive testing carriers (N=13)	p-value
Age (median, range)	63 (22-94)	63 (22-94)	68 (29-86)	59 (30-82)	0.501
Sex, female (N, %)	347 (46%)	323 (45%)	21 (66%)	3 (23%)	0.022
Race (N, %)					0.525
White	559 (74%)	521 (73%)	25 (78%)	13 (100%)	
Black/African American	146 (19%)	140 (20%)	6 (19%)	0 (0%)	
Asian	25 (3.3%)	24 (3.4%)	1 (3.1%)	0 (0%)	
Hispanic/Latino (N,%)	32 (4.2%)	31 (4.4%)	1 (3.1%)	0 (0%)	>0.999
Cancer type (N, %)					0.085
Colorectal	348 (46%)	327 (46%)	15 (47%)	6 (46%)	
Noncolorectal gastrointestinal	320 (42%)	304 (43%)	11 (34%)	5 (39%)	
Breast	35 (4.6%)	33 (4.6%)	2 (6.3%)	0 (0%)	
Genitourinary	31 (4.1%)	30 (4.2%)	0 (0%)	1 (7.7%)	
Head and neck	20 (2.6%)	16 (2.2%)	3 (9.4%)	1 (7.7%)	
Stage (N, %)					0.732
0, I, II	129 (17.2%)	120 (16.5%)	6 (19%)	3 (23%)	
III	225 (30%)	210 (30%)	12 (38%)	3 (23%)	
IV	338 (45%)	319 (45%)	12 (38%)	7 (54%)	
ECOG					0.927
0, 1, 2	600 (79%)	564 (79%)	27 (85%)	9 (70%)	
3, 4	23 (3.1%)	21 (2.9%)	1 (3.1%)	1 (7.7%)	
Unknown	134 (18%)	127 (18%)	4 (13%)	3 (23%)	
Treatment (N, %)					
Fluorouracil-based	415 (55%)	392 (55%)	15 (47%)	8 (62%)	0.610
Capecitabine-based	256 (34%)	239 (34%)	12 (38%)	5 (39%)	--
Monotherapy	225 (30%)	210 (30%)	13 (41%)	2 (15%)	0.142
Combination regimen	446 (59%)	421 (59%)	14 (44%)	11 (85%)	--
Did not start fluoropyrimidine-based treatment	86 (11%)	81 (11%)	5 (16%)	0 (0%)	--

DPYD Allele Frequency

	All patients (N=757)	DPYD wild type (N=712)	Pre-treatment testing carriers (N=32)	Reactive testing carriers (N=13)	Race
***DPYD* genotype (N, %)**					
Wild-type (*1/*1)	712 (94%)	712 (100%)	0 (0%)	0 (0%)	
Heterozygous carrier	**45 (5.9%)**	0 (0%)	32 (100%)	13 (100%)	
*1/c.1236G>A (HapB3)	23 (3.0%)	0 (0%)	16 (50%)	7 (54%)	22 White, 1 Asian
*1/c.2846A>T	8 (1.1%)	0 (0%)	5 (16%)	3 (23%)	8 White
*1/c.557A>G	7 (0.9%)	0 (0%)	7 (22%)	0 (0%)	6 Black, 1 White
*1/c.1905+1G>A (*2A)	5 (0.7%)	0 (0%)	2 (6.3%)	3 (23%)	5 White
*1/c.1679T>G (*13)	2 (0.3%)	0 (0%)	2 (6.3%)	0 (0%)	2 White

Implementation Metrics

Turnaround time (median, IQR)	Days
Overall turnaround time	6 (3-7)
Time from sample collection to receipt	1 (1-2)
Time from sample receipt to result	3 (2-6)

Timing of testing (N, %)	Patients (N=757)
Pre-treatment testing	621 (82%)
DPYD variant carrier rate	32 (5.2%)
Resulted by treatment start date	561 (90%)
Reactive testing	136 (18%)
DPYD variant carrier rate	13 (9.6%)
Collected on treatment start date	59 (43%)

Fluoropyrimidine modifications upon result return (N, %)	DPYD variant carriers (N=45)
Pre-treatment testing	32 (71%)
Dose reduced	27 (84%)
Not started[1]	5 (16%)
Reactive testing	13 (29%)
Dose reduced	9 (69%)
Discontinued[2]	1 (7.7%)
No change[3]	3 (23%)

[1]3 died before treatment started, 1 declined chemotherapy, and 1 had treatment avoided due to variant and hepatic impairment.
[2]Due to variant and grade 3 toxicities.
[3]Tolerating therapy per provider.

Of 42 variant carriers, 38 (90%) had genotype-guided FP dose reduction or discontinuation

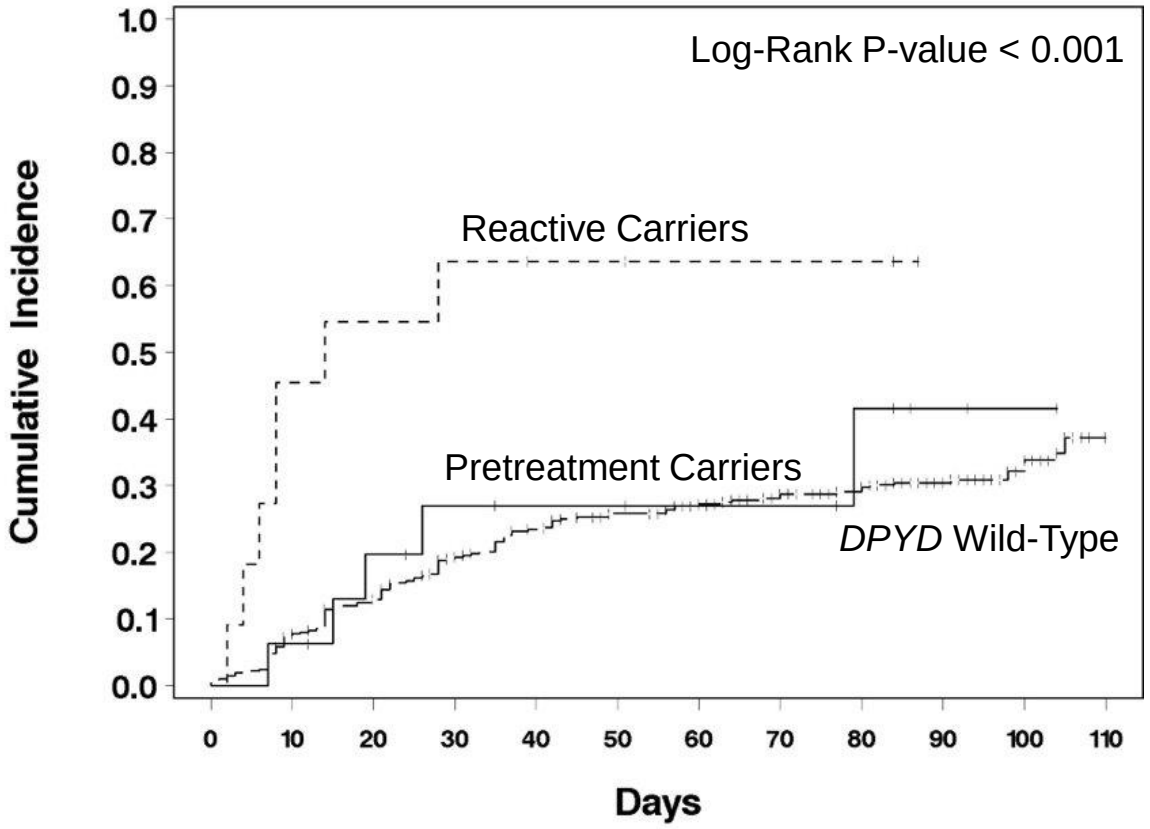
FP Dose Intensity, Grade 3+ Toxicities, and Hospitalization

	All patients (N=442)	DPYD wild-type (N=415)	Pre-treatment testing carriers (N=16)	Reactive testing carriers (N=11)	p-value
Relative dose intensity, first cycle (mean, range)	93% (26-121%)	94% (26-121%)	55% (29-89%)	95% (74-102%)	-
FP-related grade 3+ toxicity, N (%)	138 (31%)	126 (30%)	5 (31%)	7 (64%)	0.085
Hematological toxicity	66 (15%)	62 (15%)	1 (6.3%)	3 (27%)	0.277
Gastrointestinal toxicity	77 (17%)	67 (16%)	4 (25%)	6 (55%)	0.006
Hand-foot syndrome	7 (1.6%)	7 (1.7%)	0 (0%)	0 (0%)	>0.999
Other[1]	2 (0.5%)	2 (0.5%)	0 (0%)	0 (0%)	>0.999
FP-related hospitalization, N (%)	64 (15%)	53 (13%)	4 (25%)	7 (64%)	<0.001
FP-related treatment discontinuation, N (%)	41 (9.3%)	37 (8.9%)	3 (19%)	1 (9.1%)	0.281

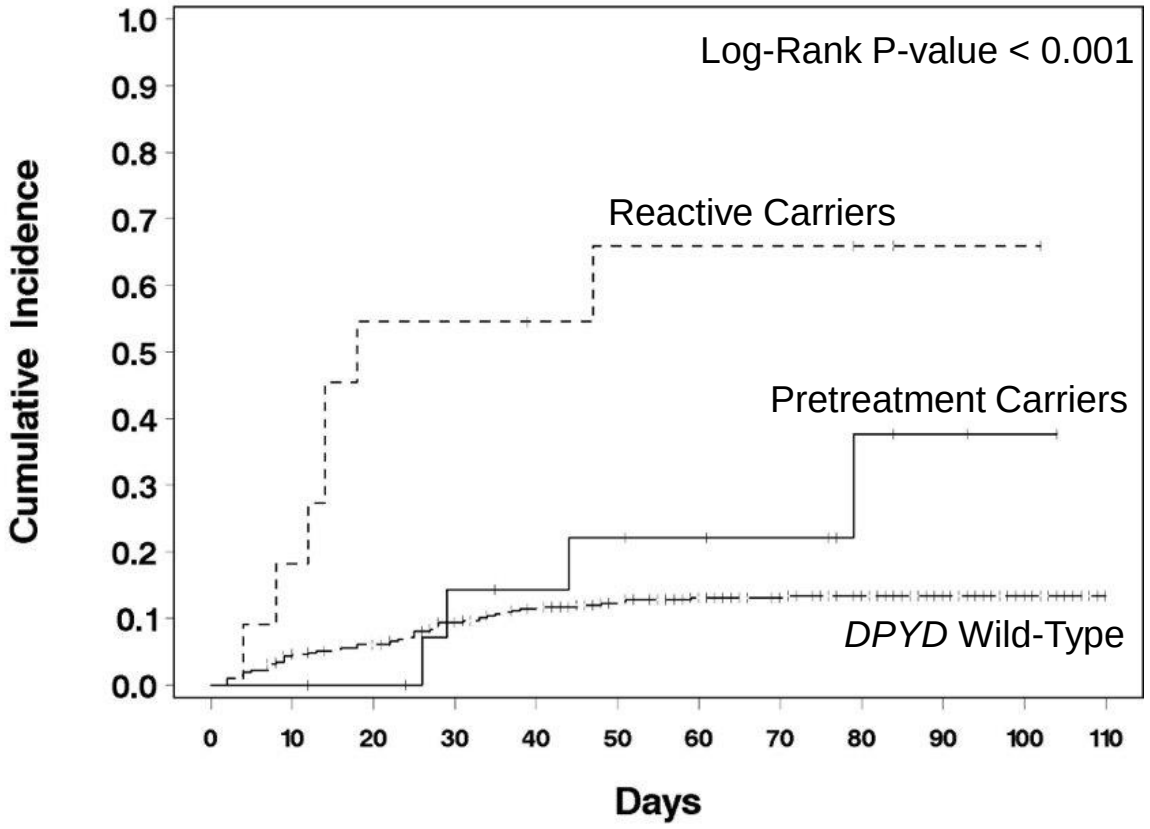
[1]Cardiovascular related events

Cumulative Incidence

Grade 3+ Toxicities



Hospitalizations

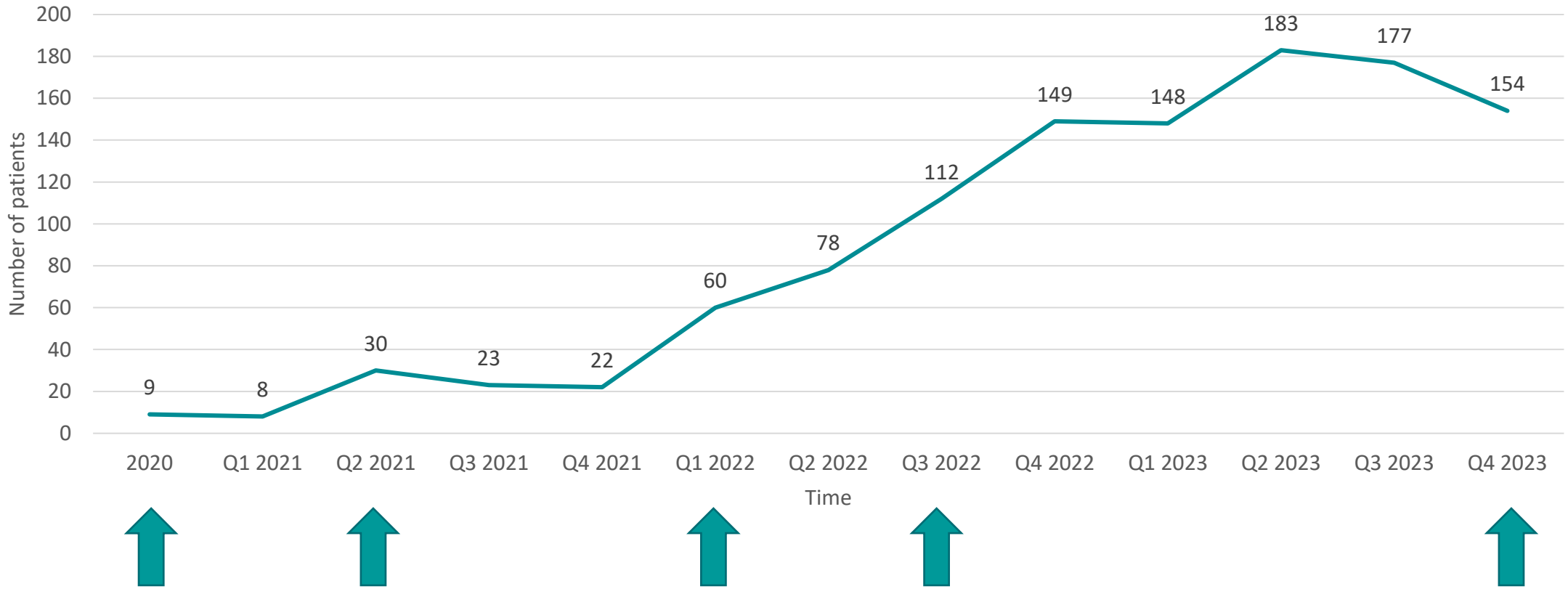


Co-variates	Grade 3+ Adverse Events (N=442)				Hospitalizations (N=442)			
	Univariate Results		Multivariable Results		Univariate Results		Multivariable Results	
	OR (CI)	p-value	OR (CI)	p-value	OR (CI)	p-value	OR (CI)	p-value
DPYD genotype category		0.092		0.130		<0.001		0.001
Wild-type vs. pre-treatment carriers	1.04 (0.36, 3.06)		1.25 (0.42, 3.74)		2.28 (0.71, 7.32)		2.02 (0.62, 6.56)	
Wild-type vs. reactive carriers	4.01 (1.15, 13.96)		3.57 (1.02, 12.49)		11.95 (3.38, 42.22)		9.59 (2.70, 34.04)	
Sex								
Male vs. female	0.84 (0.56, 1.26)	0.402	--	--	0.98 (0.58, 1.67)	0.946	--	--
Age (years)	1.00 (0.98, 1.01)	0.625		--	1.01 (0.99, 1.03)	0.507		--
Race		0.675		--		0.085		0.163
White vs. Asian	0.40 (0.09, 1.82)		--		0.39 (0.05, 3.08)		0.46 (0.06, 3.59)	
White vs. Black	1.07 (0.64, 1.80)		--		0.31 (0.12, 0.79)		0.35 (0.13, 0.90)	
White vs. Other	0.99 (0.34, 2.93)		--		NE (NE, NE)		NE (NE, NE)	
Ethnicity		0.409		--		0.887		--
Non-Hispanic vs. Hispanic	1.29 (0.53, 3.15)		--		1.32 (0.43, 4.04)		--	
Non-Hispanic vs. Other	4.52 (0.41, 50.25)		--		NE (NE, NE)		--	
Diagnosis		0.123		--		0.314		--
Non-GI vs. colorectal	1.05 (0.51, 2.17)		--		2.51 (0.74, 8.58)		--	
Non-GI vs. non-colorectal GI	1.59 (0.77, 3.29)		--		2.58 (0.75, 8.90)		--	
Stage		0.783		--		0.636		--
IV vs. 0/I/II/III	0.87 (0.57, 1.32)		--		0.77 (0.44, 1.33)		--	
IV vs. unknown	0.85 (0.38, 1.88)		--		0.89 (0.32, 2.48)		--	
ECOG		0.513		--		0.784		--
0/1 vs. 2/3/4	1.36 (0.74, 2.52)		--		1.25 (0.57, 2.73)		--	
0/1 vs. unknown	1.24 (0.71, 2.14)		--		0.88 (0.41, 1.89)		--	
Treatment Route								
Oral vs. IV	1.69 (1.09, 2.60)	0.018	--	--	1.15 (0.66, 2.00)	0.625	--	--
Treatment Regimen								
Monotherapy vs. combination	1.73 (1.11, 2.69)	0.015	1.69 (1.08, 2.65)	0.022	1.45 (0.81, 2.60)	0.210	--	--

Discussion

- Real-world analysis of U.S-based patients receiving *DPYD* genotyping demonstrated:
  - *DPYD* implementation across multiple clinic locations proved feasible
  - Almost all variant carriers received genotype-guided fluoropyrimidine modifications
  - Pre-treatment testing with genotype-guided dosing reduced severe toxicities and hospitalizations compared to reactive testing
- Study limitations
  - Performed at a single institution with in-house testing capabilities
  - Small sample size of *DPYD* variant carriers
  - Toxicity data collected from standard of care clinic notes and lab results
  - Limited to 3-month follow up, did not include survival endpoints

DPYD Testing Volume Per Quarter



1 clinic

2 clinics

Grant funding & expansion to **14** clinics

Clinical decision support alerts in EHR at all sites (8/2022)

Go-live with *DPYD* electronic ordering and billing (10/2023)

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ASHP 2023 Best Practices Award

In-house genotyping program identifies safer doses of fluoropyrimidine chemotherapies

EHR physician alert for *DPYD* testing when 5-FU or Xeloda ordered

Cheek swab sample provided by patient

Sample tested for 5 variants at in-house CLIA-certified lab

Results reported via EHR in an average of 3 days

1,200+ patients tested across 14 sites

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Clinical News From the American Society of Clinical Oncology

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Next Steps

- ❑ Expand variant coverage based on pending AMP recommendations
- ❑ Evaluate multi-gene panel testing in the same population
- ❑ Begin banking DNA to perform *DPYD* sequencing for discovery/validation
- ❑ Conduct cost analyses
- ❑ Expand testing to Wake Forest and Advocate Aurora

Acknowledgments

- **OUR PATIENTS**
- Heineman-Robicsek Foundation, Inc.
- Atrium Health Levine Cancer Institute Leadership (Derek Raghavan, MD, PhD, Founding President)
- LCI Department of Cancer Pharmacology & Pharmacogenomics
- LCI Molecular Biology and Genomics Core Laboratory
- LCI Department of Biostatistics and Data Sciences
- LCI Department of Pharmacy
- LCI Department of Solid Tumor Oncology
- Atrium Health Information and Analytics Services