CLINICAL RESEARCH

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PRINCIPAL INVESTIGATOR SIGNATURE

STUDY SPONSOR:	Penn Center for Precision Medicine Perelman School of Medicine University of Pennsylvania
STUDY TITLE:	ImpleMenting PhArmacogenetiC Testing in Gastrointestinal Cancers (IMPACT-GI)
STUDY ID	844763v 6.0
PROTOCOL VERSION	

I have read the referenced protocol. I agree to conduct the study in accordance to this protocol, in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP), and all applicable regulatory requirements and guidelines.

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Abbreviations

5-FU	Fluorouracil
ACC	Abramson Cancer Center
AE	Adverse Event
CAG	Center for Applied Genomics
CHOP	Children's Hospital of Philadelphia
CLIA	Clinical Laboratory Improvement Amendments
CDS	Clinical Decision Support
CRF	Case Report Form
DPYD	Dihydropyrimidine dehydrogenase
EHR	Electronic Health Record
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	Gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
HrQoL	Health Related Quality of Life
HUP	Hospital of the University of Pennsylvania
IB	Investigator's Brochure
ICER	Incremental Cost-Effectiveness Ratio
IDE	Investigational Device Exemption
IRB	Institutional Review Board
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NCT	National Clinical Trial
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OS	Overall survival
PCAM	Perelman Center for Advanced Medicine
PMBB	Penn Medicine Biobank
PHI	Protected Health Information
PPMC	Penn Presbyterian Medical Center
PI	Principal Investigator

PGx	Pharmacogenetic
PFS	Progression-free survival
QA	Quality Assurance
QC	Quality Control
QALY	Quality-Adjusted Life Years
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SoA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
TAT	Turnaround time
UGT1A1	Uridine diphosphate-glucuronosyltransferase isoform 1A1
US	United States
WHO / ECOG	World Health Organization / Eastern Cooperative Oncology Group

1 STUDY SUMMARY

1.1 Synopsis

Title:	ImpleMenting PhArmacogenetiC Testing in Gastrointestinal Cancers
Short Title:	IMPACT-GI
Study Description:	Pharmacogenetic (PGx) variants in the *DPYD* and *UGT1A1* genes are associated with fluoropyrimidines and irinotecan induced adverse events. However, testing for these variants is not routinely performed in clinical practice prior to the initiation of chemotherapy due to lack of a clinical assay with rapid turnaround time (TAT) and challenges in integrating genetic test results within the electronic health record (EHR). We hypothesize that providing clinicians with the ability to order rapid turnaround PGx test results along with specific dosing recommendations will increase the utilization of PGx tests to guide pharmacotherapy decisions and improve patient drug related outcomes. This is a non-randomized implementation study to determine the feasibility of establishing and integrating a PGx test into clinical care to guide chemotherapy in patients with gastrointestinal (GI) cancers. Effectiveness of the PGx-guided approach will be determined by comparing the incidence of severe treatment related adverse events to historical control group of GI cancer patients enrolled into the Penn Medicine Biobank (PMBB).
Objectives:	Implementation Aims: 1. To determine the <u>feasibility</u> of returning PGx results prior to the first dose of chemotherapy. 2. To determine the <u>fidelity</u> to the PGx guided dosing recommendations. 3. To determine the rate of testing among providers with patients eligible for testing Effectiveness Aims: 4. To determine if providing PGx test results will decrease the number of patients severe treatment related events during the first six cycles of chemotherapy.

	5. To determine if providing PGx test results will improve patient reported outcomes (PRO) during the first six cycles of chemotherapy.
Primary Endpoint:	1. The proportion of PGx test results returned prior to the first dose of chemotherapy. [Time Frame: 14 days] 2. The proportion of dose modifications made in agreement with the genotype guided dosing recommendations at the first dose. [Time Frame: 14 days] 3. The proportion of tests ordered compared to the number of patient with eligible for testing. [Time Frame: 14 days]
Secondary Endpoints:	1. The proportion of patients experiencing severe treatment related adverse events (TRAEs) over the first six cycles of chemotherapy. [Time Frame: 6 cycles] 2. The relative dose intensity of 5-FU and irinotecan dosing over the first six cycles. [Time Frame: 6 cycles] 3. Patient reported outcomes (PROs) during the first six cycles of chemotherapy. [Time Frame: 6 cycles] 4. Patient knowledge about and attitudes towards PGx. [Time Frame: 6 months] 5. The incidence of patients of African ancestry experiencing TRAEs over the first six cycles of chemotherapy. [Time Frame: 6 cycles] 6. The frequency of actionable DPYD and UGT1A1 in various ancestry groups. [Time Frame: 6 months] 7. PFS and OS in patients after genotyping [Time Frame: 6 $\pm$ 2 months] 8. The costs of PGx-guided chemotherapy [Time Frame: 6 cycles]
Study Population:	Inclusion criteria 1. Male and female subjects, 18 years or older at time of study 2. Pathologically confirmed gastrointestinal malignancy for which treatment with a fluoropyrimidine and/or irinotecan is indicated 3. Able and willing to provide informed consent 4. Willing to undergo blood or saliva sampling for PGx testing and comply with all study-related procedures 5. Life expectancy of at least 6 months Exclusion criteria 1. Prior treatment with irinotecan 2. *DPYD* or *UGT1A1* genotype already known

	3. Severe renal or hepatic impairment (or unacceptable laboratory values) 4. Women who are pregnant or breast feeding, or subjects who refuse to use reliable contraceptive methods throughout the study 5. Treating physician does not want subject to participate
Phase:	N/a
Description of Sites/Facilities	Penn Medicine sites: 1. Perelman Center for Advanced Medicine (PCAM) 2. Penn Presbyterian Medical Center (PPMC) 3. Lancaster General Hospital (LGH)
Enrolling Participants:	Approximately 500-800 participants will be enrolled across the Penn Medicine sites.
Description of Study Intervention:	Genetic: *DPYD* and *UGT1A1* genotyping The study utilizes a lab developed test (Center for Applied Genomics (CAG), Children's Hospital of Philadelphia, PA) performed in a Clinical Laboratory Improvement Amendments (CLIA) environment that provides identification of a patient's genotype determined from genomic DNA from a blood or saliva sample with a turnaround time of seven business days. The alleles identified and reported include DPYD*2A, *5, *6, *8, *9A, *10, *12, *13, rs2297595, rs115232898, rs67376798, HapB3 (rs75017182, rs56038477, rs56276561) and UGT1A1*6, *28. This is a nonrandomized, prospective, open label study. Patients with gastrointestinal cancers that will be initiated on chemotherapy with 5-fluorouracil (5-FU), capecitabine, or irinotecan will be consented to undergo the PGx test. The PGx test order will be placed by the medical oncologist; a blood or saliva sample will be obtained by the phlebotomist and sent to CAG for genotyping. Results will be returned in the Precision Medicine tab in PennChart. For patients with an actionable genotype (variants that would require a dose adjustment of 5-FU, capecitabine or irinotecan), clinical decision support (CDS) tools will alert the ordering oncologist of the PGx result and the recommended dose adjustment. The prescribing oncologist will ultimately decide the chemotherapy dose incorporating clinical and/or genetic factors.
Study Duration:	2 years

Participant Duration:	6 months

Key Roles and Study Governance

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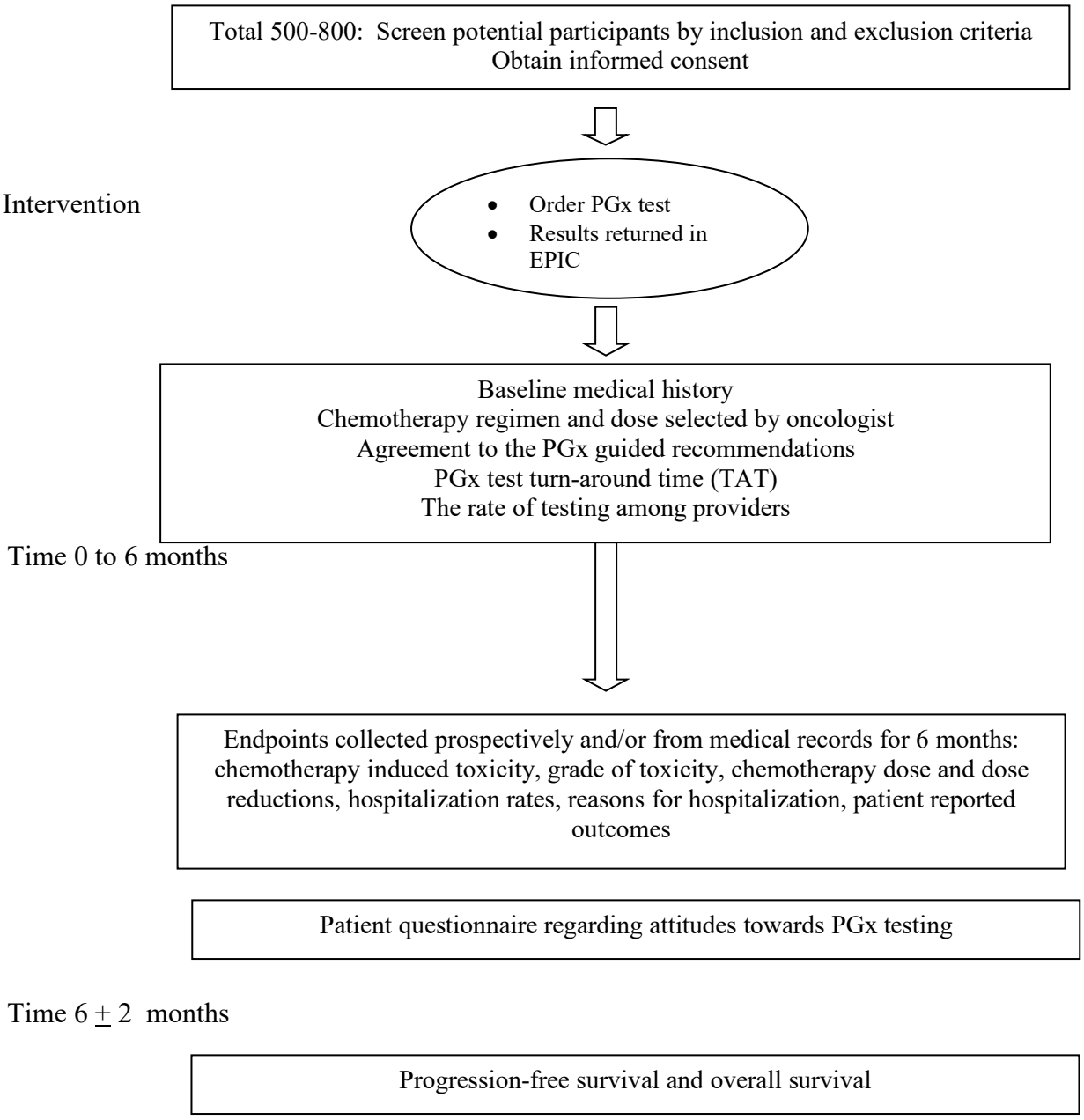
1.3 Schema


Total 500-800: Screen potential participants by inclusion and exclusion criteria
Obtain informed consent
Intervention
Order PGx test
Results returned in EPIC
Baseline medical history
Chemotherapy regimen and dose selected by oncologist
Agreement to the PGx guided recommendations
PGx test turn-around time (TAT)
The rate of testing among providers
Time 0 to 6 months
Endpoints collected prospectively and/or from medical records for 6 months: chemotherapy induced toxicity, grade of toxicity, chemotherapy dose and dose reductions, hospitalization rates, reasons for hospitalization, patient reported outcomes
Patient questionnaire regarding attitudes towards PGx testing
Time 6 ± 2 months
Progression-free survival and overall survival

2 INTRODUCTION AND RATIONALE

2.1 Study Rationale

There are known pharmacogenetic (PGx) variants in the *DPYD* and *UGT1A1* genes associated with altered drug metabolism and prolonged drug exposure resulting in drug-related adverse events from fluoropyrimidine and irinotecan therapy. Testing for these variants is not performed in routine practice due to barriers in clinical implementation. These obstacles include a lack of rapid turnaround times of genotype results to impact clinical care, a lack of a standardized format for the return of test results in the electronic health record, inexperience of clinicians on interpreting and acting on PGx information, a limited number of prospective, randomized genotype-guided clinical outcomes trials, as well as cost considerations and reimbursement. We will conduct a non-randomized implementation study to determine the feasibility of establishing and integrating a PGx test into clinical care to guide chemotherapy in patients with gastrointestinal (GI) cancers. Effectiveness of the PGx guided approach will be determined by comparing the incidence of severe TRAEsto historical control group of GI cancer patients enrolled into the Penn Medicine Biobank (PMBB).

2.2 Background

Standard first-line systemic chemotherapy for most gastrointestinal malignancies consists of a fluoropyrimidine [intravenous 5-fluorouracil (5-FU) or oral capecitabine] in combination with irinotecan, oxaliplatin, and/or targeted agents. A unique subset of patients is at an increased risk of developing severe, chemotherapy-related adverse events from fluoropyrimidines and irinotecan due to germline variants in the *DPYD* (dihydropyrimidine dehydrogenase) and *UGT1A1* (uridine diphosphate-glucuronosyltransferase isoform 1A1) genes, respectively.

Up to 30% of patients develop severe fluoropyrimidine-related toxicity, such as diarrhea, hand-foot syndrome, mucositis, and myelosuppression at standard doses.[1] These events can be fatal in 1% of treated patients.[1] Similarly, as many as 20-35% of patients treated with irinotecan may experience severe toxicities, ranging from diarrhea to myelosuppression.[2] Given the life-threatening nature of severe adverse events, hospitalizations are often indicated which result in additional resource utilization costs. Subsequently, chemotherapy-related adverse events often lead to treatment delays or discontinuations that may impact tumor prognosis.

The *DPYD* gene encodes dihydropyrimidine dehydrogenase, or DPD, the metabolic enzyme responsible for the catabolism of 5-FU and capecitabine. A partial DPD enzyme deficiency is present about 3-5% of individuals of European ancestry while complete deficiency occurs less frequently at a rate of 0.2%.[3] Four clinically relevant *DPYD* variants in European ancestry individuals and one clinically relevant *DPYD* variant in African ancestry individuals have been identified as resulting in a reduction of DPD enzyme activity of 25 to 50%.[1,3-5] These variants

include *2A (c.1905+1G>A, rs3918290), *13 (c.1679T>G, rs55886062), c.2846A>T (rs67376798), and HapB3 (c.1129-5923C>G, rs75017182; c.1236G>A, rs56038477), and c.557A>G (rs115232898). Genetic variants associated with reduced UGT1A1 enzyme activity include *UGT1A1*28 (rs8175347), *6 (rs4148323) (Table 1).[6,7]

Table 1. Actionable pharmacogenetic variants impacting response to chemotherapy

Gene	Variant				MAF		Allele Function[1,6]
	Haplotype	Nucleotide change	Protein change	rsID	EA	AA	
DPYD	*2A	c.1905+1G>A	-	rs3918290	0.008	0.003	No function
	*8	c.703C>T	R235W	rs1801266	0.0001	NR	No function
	*10	c.2983G>T	V995F	rs1801268	NR	NR	No function
	*12	c.1156G>T	E386X	rs78060119	NR	NR	No function
	*13	c.1679T>G	I560S	rs55886062	0.001	0.000	No function
	HapB3	c.1236G>A	E412E	rs56038477	0.024	0.003	Decreased function
		c. 1129-5923C>G	-	rs75017182			Decreased function
		c.483+18G>A	-	rs56276561			Decreased function
		c.557A>G	Y186C	rs115232898	0.0001	0.012	Decreased function
		c.2846A>T	D949V	rs67376798	0.004	0.003	Decreased function
UGT1A1	*6	c.211G>A	G71R	rs4148323	0.008	0.004	Decreased function
	*28	c.-41_-40dupTA(TA_7)	-	rs8175347	0.316	0.373	Decreased function

MAF minor allele frequency, EA European ancestry, AA African ancestry

The NIH-funded Clinical Pharmacogenetics Implementation Consortium, which issues evidence-based peer-reviewed guidelines on clinically actionable PGx variants, recommends a 50% dose reduction in individuals with one reduced function allele (intermediate metabolizers) and avoidance of therapy in those with two reduced function alleles (poor metabolizers).[1] Recent studies have shown that preemptive genotyping for DPYD variants can significantly reduce drug-related adverse events and improve patient safety.[8-10] In a 2018 multicenter European study, Henricks et al. demonstrated the feasibility of prospective genotype-guided fluoropyrimidine dosing in clinical practice. When comparing the safety outcomes in dose-reduced variant carriers to a historical control given the standard dose, severe toxicity was found to be higher in the standard dose population (39% vs. 23%, p=0.0013).[9] A study conducted by Kleinjan et al. in 2019 further supports the practice of *DPYD* genotype-guided dosing, demonstrating that performing initial dose reductions of capecitabine in heterozygous DPYD variant carriers followed by tolerance-based dose escalation did not lead to higher toxicity compared to patients of wild-type status (37.9% vs. 27.3%, p=0.54).[10] There are few studies directly investigating the decreased function c.557A>G variant with evidence currently limited to case reports.[5]

The FDA drug labeling for irinotecan recommends approximately a 20-40% reduction in the starting dose in UGT1A1 *28/*28 homozygous individuals (poor metabolizers) to avoid hematological toxicity.[11] The Dutch Pharmacogenetics Working Group from the Royal Dutch Pharmacists Association recommends an initial dose reduction of 30% in poor metabolizers, with subsequent dose escalation guided by patient tolerance and neutrophil counts.[12] A 2007 meta-analysis by Hoskins et al. evaluating a variety of irinotecan-containing regimens found that

*UGT1A1**28 genotyping would be likely to improve rates of severe neutropenia in homozygous patients given high doses of irinotecan (>250 mg/m^2) (OR 27.8, p=0.05).[13] A subsequent 2010 meta-analysis by Hu et al. reported that the same genotype was also associated with an increased risk of neutropenia at doses of 150 mg/m^2 to 250 mg/m^2 (RR 2.0, p<0.01) and at lower doses (<150 mg/m^2) (RR 2.4, p<0.01).[14]

DPYD genotyping has suboptimal sensitivity and positive predictive value (PPV), because DPD activity is also impacted at the post-transcriptional level, thus alternative methods have been proposed to identify those at risk for fluoropyrimidine-associated toxicity.[15] Measuring DPD activity is one option, but it is technically and logistically challenging to perform in a clinical setting.[16] Another method that is being investigated is the measurement of pre-treatment uracil.[15] DPD converts its endogenous substrate uracil into dihydrouracil (DHU). In one study, pre-treatment uracil was found to be superior to the DHU/uracil ratio as a predictor of severe toxicity and high pre-treatment uracil concentrations were strongly associated with overall severe toxicity (odds ratio 5.3, p=0.009) severe GI toxicity (OR33.7, p<0.0001), toxicity related hospitalization (OR 16.9, p<0.0001), and fatal treatment-related toxicity (OR 44.8, p=0.001).[15] In addition, genotyping *DPYD* variants together with the assessment of uracil concentrations improved the predictive accuracy of fluoropyrimidine associated toxicity. This study was done in a European population and the dynamic range of uracil needs to be confirm in a more ethnically diverse population as seen within Penn Medicine. Within the current study we will collect a pretreatment plasma sample to retrospectively examine the association of uracil concentrations with fluoropyrimidine associated toxicity within our patient population.

2.2.1 Description of intervention

The study utilizes a laboratory developed test for PGx variants in *DPYD* and *UGT1A1*, performed in a CLIA environment and provides identification of a patient's genotype determined from genomic DNA from a blood or saliva sample (See Section 6.6.1 Study Intervention Description and Appendix 14.5 Validation Reports).

3 STUDY OBJECTIVES AND ENDPOINTS

Hypothesis:

Providing clinicians with the ability to order a PGx test with a rapid turnaround time for results, along with specific dosing recommendations, will increase the utilization of PGx tests to guide pharmacotherapy decisions and improve patient drug related outcomes.

OBJECTIVES	ENDPOINTS
Primary	
1. To determine the feasibility of returning PGx results prior to the first dose of chemotherapy 2. To determine the fidelity to the PGx guided dosing recommendations. 3. To determine the rate of testing among providers with patients eligible for testing	1. The proportion of tests returned prior to the first determined dose of chemotherapy. 2. The proportion of dose modifications made in agreement with the genotype guided dosing recommendations at the first dose. 3. The proportion of tests ordered compared to the number eligible for testing.
Secondary	
1. To determine if providing PGx test results will decrease the number of patients experiencing severe treatment related adverse event (TRAEs) during the first six cycles of chemotherapy. 2. To determine the relative dose intensity of 5-FU and irinotecan administered to each patient over the first six cycles. 3. To determine if providing PGx test results will improve patient reported outcomes (PRO) during the first cycles of chemotherapy. 4. To assess patient attitudes towards PGx.	1. The proportion of patients severe TRAEs during the first six cycles of chemotherapy. 2. The relative dose intensity will be calculated as the cumulative administered dose divided by the anticipated cumulative dose over six cycles reported as mg/m^2 3. Patient reported outcomes (PROs) during the first six cycles of chemotherapy as assessed on a Likert scale. 4. Patient knowledge about and attitudes towards PGx testing as assessed on a Likert scale.
Tertiary	
1. To determine if chemotherapy-induced toxicity differs by ancestry group. 2. To determine the frequency of actionable DPYD and UGT1A1 in various ancestry groups. 3. To determine if PGx testing influences progression-free (PFS) and overall survival (OS). 4. To determine the cost-effectiveness of PGx guided chemotherapy dosing. 5. To determine the association of pre-treatment plasma uracil concentrations with severe (Grade 3 to 5) fluoropyrimidine induced toxicity with the first dose.	1. The proportion of patients experiencing a serious treatment relate adverse event during the first six cycles of chemotherapy stratified by self-reported ancestry. 2. Minor allele frequency of variants in DPYD and UGT1A1 reported by self-reported ancestry. 3. PFS and OS in patients at 6 ± 2 months. 4. Means and variances of cost in the genotype-guided group as compared to the historical control group (Costs of hospitalizations, treatment, PGx test, medical services) 5. Pre-treatment plasma uracil concentrations as measured by HPLC.

OBJECTIVES	ENDPOINTS
Optional Microbiome Substudy at PCAM only	
1. To determine whether the microbiome composition impacts the occurrence of chemotherapy-induced adverse events	1. The change in bacterial abundance as measured by operational taxonomic units (OTUs) 2. The change in bacterial composition or beta diversity as measured by weighted UniFrac distance

4 STUDY PLAN

4.1 Study Design

This is a non-randomized, prospective, open-label implementation study to determine the feasibility of establishing and integrating a PGx test into clinical care. The effectiveness aims will be assessed by comparing toxicity outcomes using a historical control group without clinical genotyping. The implementation will be deployed across cancer care sites within Penn medicine in sequential fashion, starting with PCAM followed by PPMC, and LGH.

Patients with a GI malignancy initiating chemotherapy with a fluoropyrimidine (5-FU or capecitabine) and/or irinotecan will be consented to undergo *DPYD* and *UGT1A1* genotyping, the study intervention. The PGx test order will be placed by the research coordinator and signed by the medical oncologist; a blood sample will be obtained by the phlebotomist, or saliva self-collected by the patient in clinic, and sent to CAG for genotyping. Results will be returned in the Precision Medicine tab in PennChart. For patients with an actionable genotype (variants that would require a dose adjustment of 5-FU, capecitabine or irinotecan), clinical decision support (CDS) tools will alert the ordering oncologist of the PGx result and the recommended dose adjustment. The prescribing oncologist will ultimately decide the chemotherapy dose incorporating clinical and genetic factors. The efficacy and safety of this PGx-guided approach will be determined by comparing the incidence of severe treatment related adverse events to a historical control group of GI cancer patients enrolled into PMBB.

4.2 Scientific Rationale for Study Design

This is primarily an implementation study to determine the feasibility of incorporating PGx testing as part of clinical care. Secondarily, we will use a historical control of GI cancer patients enrolled into PMBB to determine the safety and effectiveness of genotype-guided chemotherapy dosing. We performed qualitative interviews of 16 GI oncologists within Penn Medicine, and 6 (38%) stated that it would be unethical to randomize patients who may be carrying these variants to receive full-dose chemotherapy that could result in severe life-threatening toxicity. Five of the 10 oncologists supported study randomization, but as a clinician acknowledged the challenges in consenting a sufficient number of patients to undergo randomized PGx testing as this study design is not typically viewed favorably from the patient perspective.

4.3 Study Intervention

The study utilizes a laboratory developed test performed in a CLIA environment for determining PGx variants in *DPYD* and *UGT1A1* from genomic DNA isolated from a blood or saliva sample (see section 6.1.1 and Appendix 14.5 Validation Reports).

4.4 Dose Adjustments based on Genotype

Genotype-guided dose recommendations will be made according to peer-reviewed, evidence-based clinical guidelines from the NIH-funded Clinical Pharmacogenetics Implementation Consortium (CPIC) for 5-FU and capecitabine and the Dutch Pharmacogenetics Working Group (DPWG) for irinotecan (refer to Tables 2 and 3).[3,12]

Table 2. Genotype-guided dosing recommendations

Drug(s)	Gene	Genotype	Gene Activity Score*	Phenotype	Clinical Implication	Dose Recommendation
5-FU, capecitabine	*DPYD*	See Table 3 below	2	Normal Metabolizer	Patient is predicted to have a normal risk of toxicity when treated with 5-FU or capecitabine.	Initiate 5-FU or capecitabine at the standard dose.
			1.5	Intermediate Metabolizer	Patient is predicted to have an increased risk of severe toxicity when treated with 5-FU or capecitabine.	Reduce starting dose by 50% followed by dose titration based on toxicity. **5-FU Bolus:** if standard dose is 400 mg/m^2, consider 200 mg /m^2 **5-FU CIV:** If standard dose is 2400 mg/m^2, consider 1200 mg/m^2 **Capecitabine:** If standard dose is 1000-1250 mg/m^2, consider 500-625 mg/m^2

			1	Intermediate Metabolizer	Patient is predicted to have an increased risk of severe toxicity when treated with 5-FU or capecitabine.	Reduce starting dose by 50% followed by dose titration based on toxicity. **5-FU Bolus:** if standard dose is 400 mg/m^2, consider 200 mg /m^2 **5-FU CIV:** If standard dose is 2400 mg/m^2, consider 1200 mg/m^2 **Capecitabine:** If standard dose is 1000-1250 mg/m^2, consider 500-625 mg/m^2
			0.5	Poor Metabolizer	Patient is predicted to have an increased risk of severe toxicity when treated with 5-FU or capecitabine.	Avoid use of 5-FU or capecitabine-based regimens. If alternative agents not considered a suitable option, 5-FU should be administered at a strongly reduced dose (i.e. <25% of normal starting dose) with early therapeutic drug monitoring.
			0	Poor metabolizer	Patient is predicted to have an increased risk of severe toxicity when treated with 5-FU or capecitabine.	Avoid use of 5-FU or capecitabine-based regimens.
Irinotecan, Liposomal irinotecan	*UGT1A1*	*1/*1	N/A	Normal Metabolizer	Patient is predicted to have a normal risk of toxicity when treated with irinotecan.	Initiate irinotecan at the standard dose.
		*1/*28 *1/*6	N/A	Intermediate Metabolizer	Patient is predicted to have a normal risk of toxicity when treated with irinotecan.	Initiate irinotecan at the standard starting dose.
		*28/*28 *6/*6 *6/*28	N/A	Poor Metabolizer	Patient is predicted to have an increased risk of severe toxicity when treated with irinotecan.	Irinotecan: Reduce starting dose by 30%. If tolerated, increase dose based on the neutrophil count. Liposomal irinotecan: Initiate 50 mg/m^2 IV over 90 minutes. Increase to 70 mg/m^2 IV as tolerated in subsequent cycles.

Table 3. *DPYD* activity scoring system

DPYD Allele/rsID	*Activity Value	Allele Function
*1	1	Normal
*2A	0	None
*5	1	Normal
*6	1	Normal
*8	0	None
*9A	1	Normal
*10	0	None
*12	0	None
*13	0	None
HapB3 (rs75017182, rs56038477, rs56276561)	0.5	Decreased
rs115232898	0.5	Decreased
rs67376798	0.5	Decreased
rs2297595	1	Normal

Calculating a DPYD activity score

1. An activity score is used to interpret DPYD genetic test results and assign phenotypes.
2. Each *DPYD* variant allele is assigned a value according to its enzyme function: 1 for normal function, 0.5 for decreased function, and 0 for no function (or minimal DPD activity). The Allele Functionality table can be found at https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd to assign values to alleles.
3. The activity score is then calculated as the sum of the two *DPYD* variants with the lowest variant activity score to correspond to a phenotype.

Example: A patient's *DPYD* PGx test results are reported as *DPYD* *1/*2A. The *1 allele has a value of 1 and *2A allele has a value of 0, so the sum of these would yield an activity score of 1. This patient would then be classified as being an intermediate metabolizer.

4.5 Plasma uracil measurements

A 5 mL venous blood sample for measurement of plasma uracil will be collected in EDTA tubes from during the baseline visit if time and resources permit. After collection the blood sample will be kept on ice (2-4 °C) and processed to plasma in a timely fashion (processing to begin within 1 hour of sample procurement) as described below under section 7.7.

4.6 Optional Microbiome Substudy

Participants consenting to the optional microbiome substudy will provide two stool samples, one at baseline and a second sample anytime during the 6-month study period. Participants will be provided with collection materials, packaging and instructions for collecting the stool at home and bringing it to usual clinic visits.

5 STUDY POPULATION AND DURATION OF PARTICIPATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Able and willing to provide informed consent
2. Male or female, aged 18 years or older at the time of study initiation
3. Pathologically confirmed gastrointestinal malignancy for which treatment with a fluoropyrimidine and/or irinotecan is indicated
4. Willing to undergo blood or saliva sampling for PGx testing and comply with all study-related procedures
5. Life expectancy of at least 6 months

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

Exclusion criteria

1. Prior treatment with irinotecan
2. *DPYD* or *UGT1A1* genotype already known
3. Severe renal or hepatic impairment (or unacceptable laboratory values), including:
    - Neutrophil count of $<1.5 \times 10^9$/L, platelet count of $<100 \times 10^9$/L
    - Hepatic function as defined by serum bilirubin >1.5 x upper limit of normal (ULN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) >2.5 x ULN, or in case of liver metastases ALT and AST>5 x ULN
    - Renal function as defined by serum creatinine >1.5 x ULN, or creatinine clearance <60 ml/min (by Cockcroft-Gault Equation)
4. Women who are pregnant or breast feeding, or subjects who refuse to use reliable contraceptive methods throughout the study
5. Treating physician does not want subject to participate

5.3 Screen Failures

Screen failures in this study will be defined as participants who meet criteria for study enrollment but decline to participate or the treating oncologist declines participation of their patient in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, and eligibility criteria.

5.4 Participant recruitment

Approximately 1000 cases of GI cancer are treated annually at PCAM, 150 cases at PPMC, and 200 at LGH. Roughly 100 unique patients receive fluoropyrimidine and/or irinotecan-based chemotherapy regimen in a typical month at the PCAM GI cancer clinic. Based on the frequency of *DPYD* and *UGT1A1* variants and the number of GI cancer patients seen within Penn Medicine, we anticipate that testing 500-800 patients in this multisite study would result in 45-70 patients with the actionable genotypes (see Table 4). Approximately 20% of the GI cancer population at Penn Medicine is of African ancestry. All recruitment will be done through the Penn cancer centers and treating oncologists. Clinic schedules will be screened by the clinical research coordinator for new patients with GI cancer being evaluated for treatment. Prior to the scheduled visit, the research coordinator will notify the treating oncologist about potential eligible participants and remind them of the study. The treating oncologist will discuss the study with the patient during the evaluation visit and consent will be obtained by the treating GI oncology provider (physician or advanced practice provider) or the research coordinator. This will occur in person or by remote consent. Patients will be given a copy of the official informed consent form and an opportunity to ask questions. Patients will be given sufficient time to consider participating in the trial.

Participants who complete the patient questionnaire regarding attitudes towards PGx testing will receive a $25 gift card. The Greenphire ClinCard will be used to disburse funds and mailed to the patient.

Women of childbearing potential who are pregnant or breastfeeding will not be recruited into this study due to the risk of fetal harm caused by exposure to 5-FU, capecitabine, or irinotecan and the potential for serious adverse reactions in breastfed infants. A pregnancy test is not routinely performed in these patients at Penn Medicine thus will not be included as a study procedure.

Table 4. Anticipated study initiation and estimated participant accrual across sites

Calendar year **Site**	Q1 2021	Q2 2021	Q3 2021	Q4 2021	Q1 2022	**Site totals**
PCAM (20/mo)	60	60	60	60		**240**
PPMC (10/mo)		30	30	30	30	**120**
LGH (20/mo)		60	60	60	60	**240**
Total						**600**

5.5 Duration of study participation

Total involvement for each participant will be 6 months.

5.6 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Appendix Section 14.1.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

This study will utilize a laboratory developed test for PGx variants in *DPYD* and *UGT1A1*performed in a CLIA-approved environment. Samples will be genotyped using the Infinium Global Screening Array v2 (Illumina, San Diego, CA), a genome-wide genotyping array that contains PGx variants. Genotyping will be performed on Illumina's iScan System at CAG at CHOP. The *DPYD* SNPs of interest *2A (rs3918290), *5 (rs1801159), *6 (rs1801160), *8 (rs1801266), *9A (rs1801265), *10 (rs1801268), *12 (rs78060119), *13 (rs55886062), rs2297595, rs115232898, rs67376798, HapB3 (rs75017182, rs56038477, rs56276561) and UGT1A1 SNPs *6 (rs4148323) and *28 (rs8175347) will be extracted from the array data.

The *UGT1A1**28 SNP contains a (TA) tandem repeat. Samples will undergo a PCR-based assay for amplification and fragment analysis. In the validation report, thirteen samples were processed with a fragment analysis assay and compared to previous results obtained using next-generation sequencing (NGS) to determine concordance with TA repeat number in the UGT1A1 gene. All samples were concordant with the previous results, rendering the assay to be highly sensitive, specific, reproducible, and repeatable. Sanger sequencing was used to confirm the *DPYD* and *UGT1A1**6 SNPs. All results were concordant showing 100% repeatability and reproducibility.

6.1.2 Dosing and Administration

Dosing for 5-FU, capecitabine and irinotecan will be recommended according to the patients' *DPYD* and/or *UGT1A1* genotype as indicated in Table 2 in Section 4.4.4. Ultimate dosing decisions will be determined by the treating oncologists according to their best clinical judgment.

6.2 Study Intervention Compliance

Compliance to the PGx testing will be determined by tracking test orders in PennChart. Central laboratory records will be reviewed to determine time of the sample acquisition, time of sample receipt in the central lab, time receipt in the genotyping facility (e.g. CAG), and time of results posted within PennChart. Test turnaround time will be determined based on the time from sample acquisition to posting of results in PennChart.

6.3 Discontinuation of Study Intervention

If the patient and/or treating oncologist wish to discontinue the PGx test and the sample as already been collected, the patient sample will be discarded. Participants will not be contacted for surveys.

6.4 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request, without prejudice to their medical care, and are not obliged to state their reasons. The study investigator may discontinue or withdraw a patient from the study at any time for the following reasons:

- Pregnancy
- Patient transfers care outside of Penn Medicine
- Treating oncologist wishes patient to withdraw

The reason for participant discontinuation or withdrawal from the study will be recorded on the patient's Case Report Form (CRF). Subjects who sign the informed consent form but do not undergo genotyping may be replaced. Subjects who sign the informed consent form and do undergo genotyping, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

6.5 Lost To Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for completion of scheduled chemotherapy infusion appointments. There are no study specific visits. Data is collected at the time of usual clinic visit and from the medical record

The following actions will be taken if a participant fails to return to the clinic for a scheduled visit/follow up:

- The research coordinator will contact the treating oncologist and ensure the reason for not returning is not a (serious) adverse event ((S)AE)
- If patient wishes to discontinue in the study, an attempt will be made to establish that the true reason is not an AE (bearing in mind that the patient is not obligated to share his/her reasons).
- If treatment is prematurely discontinued, the primary reasons for discontinuation must be recorded in the patient's file and all efforts will be made to complete and report he observations as thoroughly as possible.

- A complete final evaluation following the patient's withdrawal will be made, and any AEs will be followed up until resolution or a period of 30 days from the last dose of chemotherapy has elapsed, whichever is shorter.

7 STUDY ASSESSMENT AND PROCEDURES

7.1 Informed Consent (Baseline)

Eligibility for the study test will be determined by study personnel who will screen the clinic schedule. Study personnel will inform the treating oncologist of eligible patients by email or in person on the day of the evaluation visit. Participants will be consented in-person during the same visit or via remote consent (telephone/electronic). Blood or saliva sample will be collected on the same day if possible to ensure results will be returned prior to the first dose of chemotherapy.

7.2 Genotyping (0-14 days)

Study personnel will place an order for the PGx test in PennChart to be signed by the medical oncologist. If saliva is not self-collected by the patient in clinic, the phlebotomist will obtain a blood sample that will be sent to the Center for Applied Genomics (CAG) at the Children's Hospital of Philadelphia by courier.

7.3 Implementation Metrics (0-14 days)

The following information if available will be collected to determine the feasibility of PGx test implementation and agreement to the PGx guided dosing recommendations:

- Dates of sample collection, return of PGx test within PennChart, and timing of first dose of chemotherapy
- Intended and prescribed doses of chemotherapy
- Reasons for not adhering to the PGx guided recommendations
- The proportion of tests ordered compared to the number eligible for testing.

7.4 Baseline clinical data collection

The following information will be obtained at screening and from the patient medical record if performed as part of usual care and available in the medical record:

- Signed informed consent form
- Inclusion and exclusion criteria
- Demographic data: age, gender, race/ethnicity
- Cancer history: GI tumor type, stage, previous cancer treatments, number of lines of therapy
- Routine physical examination: ECOG performance status (see Appendix 14.2), height (cm), weight (kg)
- Vital signs: heart rate, blood pressure
- Hematology: hemoglobin, hematocrit, white blood cell count, ANC, neutrophils, eosinophils, basophils, lymphocytes, monocytes, platelets

- Clinical chemistry: sodium, potassium, calcium, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, total bilirubin, albumin
- Creatinine clearance (using Cockcroft-Gault formula)
- Concomitant medications (including dose, unit, frequency, route of administration and indication)

7.5 Data collection at each chemotherapy infusion (time 0 to 6 cycles)

The following information will be assessed at each chemotherapy visit by study personnel or extracted from the medical record with each chemotherapy treatment during the first six cycles if performed as part of usual care and available in the medical record.

- Routine physical examination: height (cm), weight (kg), calculated body mass index (BMI)
- Vital signs: heart rate, blood pressure
- Hematology: hemoglobin, hematocrit, white blood cell count, ANC, neutrophils, eosinophils, basophils, lymphocytes, monocytes, platelets prior to treatment
- Clinical chemistry: sodium, potassium, calcium, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, total bilirubin, albumin
- Creatinine clearance (using Cockcroft-Gault formula)
- Concomitant medications (including start date, dose, unit, frequency, route of administration and indication)
- Chemotherapy course: dates of treatment, dose of each treatment for first 6 cycles
- Adverse events: date of reported treatment-related symptoms, date of emergency department (ED) visits and/or hospitalizations (if applicable), clinical course and symptom duration, ED/hospitalization medical billing information. Severe TRAEs are defined as those requiring treatment in the hospital, ED, or Oncology Evaluation Center (OEC).
- Serial questionnaires assessing patient reported outcomes (PROs) for symptoms associated with to adverse events will be prospectively collected by the study team at the time of each treatment for the first 6 cycles (see data collection tool in Appendix 14.6)

The following information will be extracted from the medical records and/or tumor registry at the 6-month ($\pm$ 2 months) follow-up period if performed as part of usual care and recorded in the medical record.

- Overall patient survival (duration of patient survival from time of treatment initiation)
- Progression free survival (time from treatment initiation until disease progression or worsening)
- Information regarding tumor response from computed tomography (CT) scans will also be collected as available. This is expected to be classified by the treating oncologist as complete response (CR), partial response (PR), stable disease (SD), or progressive

disease (PD) per response evaluation criteria in solid tumors (RECIST) guideline version 1.1.

7.6 Patient Survey on attitudes towards pharmacogenetic testing (0-6 months)

Patients will complete an electronic survey via an emailed/texted link in RedCap to assess their knowledge and attitudes towards pharmacogenetics (Appendix 14.7). A paper copy will be made available for those without computer access. Patients will be contacted about the survey three times, after which time the survey will be recorded as missing data.

7.7 Plasma uracil assay (PCAM only)

If the patient is willing, a plasma sample will be obtained during the baseline visit as described in 4.5 above. Samples will be marked with a coded study-specific patient de-identifier. Blood samples will be centrifuged at 1500 g for 10 minutes in a refrigerated centrifuge (2-4°C) and plasma aliquots separated. Plasma will be stored in -80^{0}C freezers until analysis. Plasma uracil concentrations will be determined by UPLC-MS/MS (ultra-high performance liquid chromatography – dual mass spectrometry), using a previously published and validated assay with any necessary minor modifications in collaboration with the clinical pharmacology lab at the Norris Cotton Cancer Center at Dartmouth-Hitchcock.[17]

7.8 Optional Microbiome study (PCAM only)

If participants consent to this portion of the study, participants will be provided a stool collection kit during the baseline visit. Participants will be instructed on home stool collection methods and will be required to collect a stool sample and bring it with them to their first chemotherapy infusion visit and one other clinical visit. Study staff will provide the participant with collection materials, packaging and instructions for collecting the stool at home and bringing it to the study visits (Appendix 14.8). Participants will be provided with ice packs and container in which to store the sample. Immediately after voiding the sample, the participant will rate the stool on the Bristol Stool Chart (Appendix 14.8.1). The study coordinator will bring this stool sample to the study lab on the 11th floor Smilow Center for Translational Research (SCTR). Lab personnel will aliquot the stool sample into 4 spoon-top vials and stored at -80ºC for microbiome sequencing and fecal metabolomics.

8 STATISTICAL PLAN

8.1 Sample size

For the implementation aims, the sample size is based on average GI oncology clinical volume and anticipated number of patients that are eligible for testing. We anticipated testing 500-800 patients during the course of the study at the three Penn Medicine sites. No hypothesis testing will be performed.

For the effectiveness aims, we hypothesize that the chemotherapy-related severe TRAEs will decrease from 60% in the variant carriers receiving full dose chemotherapy in the historical control group enrolled into the PMBB to 30% in variant carriers receiving genotype guided chemotherapy.[18] The following table presents the power for different sample sizes per group and different expected proportions experiencing severe TRAEs in the PGx group, based on two-sided Fisher's exact test at the alpha level of 0.05 (Table 5). Based on the frequencies of *DPYD* and/or *UGT1A1* in the population (~10%), testing 500 patients in each group will result in ~50 patients with actionable genotypes.

Table 5. Power estimate for the reduction in Severe TRAEs with PGx testing

Toxicity rate N in each group with actionable variants	39% in PGx	35% in PGx	30% in PGx
18	17.8%	24.8%	35.6%
36	32.3%	45.8%	63.6%
48			**80%**
54	49.4%	66.3%	83.9%
70		**80%**	
72	67.4%	82.9%	
97	**80%**		

8.2 Overview of statistical methods

Descriptive statistics (mean, SD, median, interquartile range, range, counts, and percentage) will be used to describe and compare (t-test or rank sum test for continuous variables and Fisher's exact test for categorical variables) baseline characteristics between the historical control group and PGx arms.

8.2.1 Implementation Endpoint(s):

a. We will report the number and proportion of tests returned prior to the first dose of chemotherapy.

b. We will report the proportion of dose modifications made in agreement with genotype-guided dosing recommendations at the first dose.

c. The proportion of tests ordered compared to the number eligible for testing.

8.2.2 Secondary Endpoint(s):

1. Clinical endpoints

a. The proportion of patients experiencing severe TRAEs will be compared in the PMBB historical control group vs. the PGx group using Fisher's exact test.

Subgroup analyses will be performed by tumor type.

b. The relative dose intensity (RDI) will be calculated for 5-FU, capecitabine or irinotecan as the cumulative administered dose divided by the anticipated cumulative dose over the first 6 cycles. RDI will be compared in the PMBB historical control group vs. the PGx group using linear regression adjusted for sex, race, BMI, tumor type, cancer stage, line of therapy and ECOG performance status.

Subgroup analyses will be performed by tumor type.

2. Patient reported outcomes

Patient reported outcomes as assessed on a Likert scale will be reported as means (SD) and compared between the PMBB historical control group vs. the PGx group using linear regression adjusting for age, sex, race, tumor type, cancer stage, BMI, line of therapy, and ECOG performance status.

3. Patient attitudes toward PGx testing

Patient knowledge and attitudes towards PGx testing will be assessed on a Likert scale will be reported as means (SD). We will compare results by sex, race, tumor type, and insurance status by using linear regression. (See survey in Appendix 14.7)

8.2.3 Tertiary Endpoint(s):

1. Clinical endpoints in minority populations

   The proportion of patients experiencing severe TRAEs will be compared in the PMBB historical control group vs. the PGx group using Fisher's exact tests, stratified by race.

2. Minor allele frequencies will be reported by ancestry for the DPYD and UGT1A1 genes.

3. Medical services utilization and costs

   We will obtain data from Data Analytics Center (DAC) on service utilization and costs. These will include: number of physician visits; number of emergency department visits; number of hospitalizations; length of hospital stay; number of emergency department and hospital admissions associated with drug-related adverse events; DPYD and UGT1A1

genetic testing costs; physician visit cost; emergency department visit costs; hospitalization costs; inpatient and physician-administered medication costs; and total costs. Care Everywhere will be utilized to obtain information if patients received care outside Penn Medicine. Additionally, medical records will be requested for admissions that occur outside of Penn Medicine.

We will compare utilization and costs incurred by the PMBB usual care group with utilization and costs incurred the PGx group using Poisson regression methods (for number of visits), and linear regression methods with appropriate transformations (e.g,, log-transform, winsorization) to account for skewness in utilization and cost distributions.

4. Survival Analysis

   Overall survival and progression free survival at approximately six months will be compared between the PMBB historical control group vs. the PGx group by use of Kaplan-Meier estimators and log-rank tests. Cox proportional regression analysis will be performed to adjust for clinical covariates including age, sex, race, tumor type, cancer stage, BMI, and ECOG performance status.

5. Pre-treatment uracil and fluoropyrimidine associated toxicity

   First, we will examine the range of pre-treatment uracil concentrations with the association of severe toxicity to determine if there is a cut-off whereby toxicity occurs. Previous studies have proposed uracil > 16g/ml as highly predictive for severe toxicity, but we will validate this cut-off in our patient population.[15]We will also examine whether the pre-treatment uracil levels vary in *DPYD* variant carriers. Second, we use logistic regression models to determine the risk of severe toxicity including a binary factor for uracil concentration (above and below cut-off), with adjustment for age, sex and treatment regimen.

8.3 Microbiome analysis (optional sub-study)

From the microbiome sequencing, we will obtain tables of OTUs, which will be used to determine the relative abundance of taxa in participants with severe TRAEs vs. those without severe TRAEs. Weighted UniFrac distance (distance measurement which incorporates phylogenetic relationship, weighted by abundance)[19,20] is the outcome we will use to examine beta diversity. We will compare the change in beta diversity pre and post- chemotherapy. The overall difference of the microbiome composition will be testing using permutation distance-based multivariate analysis of variance (PERMANOVA).[21] Non-parametric rank tests will be used to detect differences in taxon abundance pre- and post chemotherapy and between participants with severe TRAEs vs. those without severe TRAEs . Differential abundance of specific taxa will be assessed using nonparametric rank tests or generalized linear mixed effects models. To adjust for potential confounders and to evaluate the association between clinical data (e.g. age, sex, body mass index, chemotherapy, tumor type, ECOG status) and microbial

community profiles, we will use the microbiome regression-based kernel association test (MiRKAT).[22] The association between changes in microbial composition with clinical characteristics (sex, race, age, BMI, diet) and outcome (toxicity) will be assessed using multivariate modeling. The generalized linear models will be selected when within vs. between-subject effects will be characterized. False discovery rate (FDR) of 5% will be applied to account for multiple comparisons.

9 SAFETY AND ADVERSE EVENTS

9.1 Adverse Events and Serious Adverse Events

9.1.1 Definition of Adverse Events (AE)

An adverse event (AE) is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related. AEs in clinical investigation will include those associated with the study intervention (the PGx test). Adverse events associated with chemotherapy treatment will be collected as study outcomes, not as study-related adverse events.

Anticipated AEs may include incidental findings related to the PGx test results and the sharing of information to patients. The study team will communicate incidental findings to the treating oncologist. The oncologist may choose to return these findings to patients if pertinent for clinical care (see Known Potential Risks in Section 10.2.1).

9.1.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Events (SAE)

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that, in the view of the investigator is:

- fatal
- life-threatening at the time of the event
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event when the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious. Likelihood of the SAE being attributed to the PGx testing will be documented.

Unanticipated Adverse Device Effect (UADE)
A UADE is any serious adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects. Likelihood of the UADE being attributed to the laboratory developed test will be documented.

9.1.3 Classification of an Adverse Event

AEs in clinical investigation will include AEs associated with the PGx test since the chemotherapy agents that will be administered are standard of care. These will primarily be related to HIPAA issues and unexpected findings.

9.1.3.1 Relationship to Study Intervention

All adverse events (AEs) will have their relationship to the PGx test assessed by study personnel and/or the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- Related – The AE is known to occur with the PGx test, there is a reasonable possibility that the PGx test caused the AE, or there is a temporal relationship between the PGx test and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the PGx test and the AE.

- Not Related – There is not a reasonable possibility that the administration of the PGx test caused the event, there is no temporal relationship between the PGx test and event onset, or an alternate etiology has been established.

9.1.4 Time Period and Frequency for Event Assessment and Follow-Up

Safety will be assessed by study personnel at the time of PGx test resulting for subjects. Chemotherapy-induced toxicity outcomes will be documented using defined parameters described in section 7.5.

As much as possible, each adverse event or follow-up information will be evaluated to determine:

1. Description of adverse event
2. Date of occurrence
3. Expectedness to study intervention (PGx test) – [Unexpected (Yes/No)].
4. Impact on patient care – [Patient informed (Yes/No)]

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in patient care.

9.1.5 Adverse Event Reporting

AEs encountered during the study will be documented in the patient's file and reported on the Case Report Form (CRF). Likelihood of the AE being attributed to the PGx test will be documented.

The information that will be recorded in the patient's file consists of:

- Description of the event
- Date of the event
- Impact on patient care

Reporting to the IRB will be done in accordance to the ***Penn IRB definition of reportable events and reporting timelines***.

Reporting Period

Adverse events will be reported from the time of informed consent until study completion.

9.1.6 Serious Adverse Event Reporting

An SAE must be reported to the study investigators by telephone within 24 hours of the event. The investigator will keep a copy of this form on file at the study site. Report SAEs by phone to:

Sony Tuteja, PharmD
Mobile- (484)-431-1002

In the event that Dr. Tuteja cannot be reached, report SAEs to:

Ursina Teitelbaum, MD
Mobile- (215) 796-7413

At the time of the initial report, the following information should be provided:

- Study Name
- Participant number
- A description of the event
- Date of onset
- Current status
- The reason the event is classified as serious
- Investigator assessment of the association between the event and the PGx test

Within the following 48 hours, the investigator must provide further information on the SAE in the form of a written narrative. Significant new information on ongoing SAEs should be provided promptly to the study investigator.

Reports of all SAEs (including follow-up information) must be submitted to the Ethics Committee (EC)/Investigational Review Board (IRB) within 10 working days, unless the SAE involves a death, which must be reported within 3 days. Copies of each report and documentation of EC /IRB notification and receipt will be kept in the Clinical Investigator's binder.

9.1.7 Reporting of Pregnancy

Pregnancy, in and of itself, is not regarded as an AE unless there is suspicion that study drug or process may have interfered with the effectiveness of a contraceptive medication or method. If a patient inadvertently becomes pregnant while on treatment, the patient will immediately be removed the study and the study investigator will be immediately notified. The outcome of the pregnancy will be reported as a SAE or case of death, spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

9.2 Unanticipated Problems

9.2.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent EC/ IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

10.2 Risk/Benefit Assessment

10.2.1 Known Potential Risks

Blood will be drawn from all participating patients for determining the genotype prior to start of chemotherapy. Risks of venipuncture include possible bruising, infection at the site, and in rare cases, fainting. These risks are minimized by using trained personnel. The impact on clinical oncologic outcomes is unknown. Chemotherapy dose adjustments will be performed by the treating oncologist according to his or her clinical judgment.

There can also be a risk in knowing genetic information. New health information about inherited traits that may affect participating patients and or their blood relatives may be found during the research study. DPD deficiency is an autosomal recessive disease; carriers usually do not have related health problems, but they do have an increased risk of complications when treated when fluoropyrimidine therapy.[23] *UGT1A1* plays a role in the metabolism of bilirubin and is associated with hereditary hyperbilirubinemia syndromes. The **28* variant is a common cause of Gilbert syndrome.[6] Individuals with Gilbert syndrome may experience transient elevations in unconjugated plasma bilirubin in response to various triggers (e.g., fasting, infection, or medications). Genotypes most implicated in Gilbert syndrome are UGT1A1*28/*28 and UGT1A1*6/*6. Crigler-Najjar syndrome type I is very rare and results from deleterious

UGT1A1 mutations that results in hyperbilirubinemia and occur early in childhood therefore the PGx testing is unlikely to result in incidental findings. However, identification of a heterozygous state may have implications for prenatal genetic counseling. The treating oncologist may choose to return these incidental findings if pertinent for clinical care. For the purposes of this current study, variants in the *UGT1A1* gene will be interpreted to guide irinotecan drug dosing.[24]

Additionally, there is the risk of loss of privacy with storing the health and genetic data of participating patients. Very rarely health or genetic information could be misused by employers or insurance companies; however, in such events, patients may have difficulty finding or maintaining a job or insurance. Laws such as the federal Genetic Information Non-Discrimination Act (GINA) prohibit employers and health insurers from discriminating against individuals based on their genetic information. However, GINA does not protect against life insurance or long-term care insurance.

10.2.2 Known Potential Benefits

The potential benefit to study participants is having their chemotherapy doses tailored to their genetic profile so as to avoid severe life-threatening toxicities. This may prevent hospitalizations due to chemotherapy-associated toxicities and improve quality of life for patients. The benefits to the health system include decreased hospitalizations and decreased costs in care.

10.2.3 Assessment of Potential Risks and Benefits

The study is considered low risk since the medications of interest in the study are FDA-approved for clinical use in GI cancer treatment and dose adjustments are commonly performed according to the patient's tolerance and clinical laboratory values. The study will provide valuable information on the best methods for incorporation PGx testing in clinical care to prevent serious adverse events.

10.3 Informed consent

All participants for this study will be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a participant, using the EC/IRB-approved consent form, must be obtained before that participant is submitted to any study procedure. This consent form must be signed by the participant or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. Potential subjects and the investigator or a member of her designated research staff will review and sign the informed consent form during the baseline visit in the GI cancer clinic. As informed consent is an ongoing process, any new information that affects a person's willingness to continue with the trial or risk profile that is received after the trial has been initiated will be provided to all

subjects, whether participation has been completed or is ongoing. In order to avoid undue influence or coercion, all subjects will be treated equally.

10.4 Confidentiality and Privacy

Information about study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed participant authorization informing the participant of the following:

- What protected health information (PHI) will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research participant to revoke their authorization for use of their PHI.

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization.

All data related to this trial will be recorded using the patients' assigned unique study number. Data will be reported only in a confidential manner such that the personal identity of any subject will not be identifiable. All study data will be maintained under a double locked system, such as a locked closet within a locked office or on a password protected computer in a locked office. At the end of the study these data will be electronically archived on a password protected computer or other electronic storage device.

10.5 Future Use of Stored Data

Data collected for this study will be entered into a RedCap database and analyzed and stored within a secure research database housed within the BioMedical Informatics Consortium (BMIC) Secure Computing Environment. All PHI within the secure research database will be encrypted. Quantifiable data are linked to subjects within the research database using an internal patient identifier not derived from any subject information.

The research database will be accessible only by the PI and designated research staff as described to the IRB. Within the research database, designated staff will be able to link data from the electronic medical record to data scanned from survey forms.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified genome-wide genotyping data (e.g. VCF files) generated at CAG will be

stored within Penn Medicine Biobank secured servers with the goal of sharing of data with Penn Medicine Researchers after appropriate approvals. These data could be used to research the genetic causes of medication response and adverse events to medication, risk for various diseases such as cancer, cardiovascular disease and diabetes, its complications and to improve treatment. The PMBB will also be provided with a code-link that will allow linking the genetic data with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have data stored for future research.

10.6 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.7 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

10.8 Records retention

Study documents and records will be retained for at least 2 years after the last participant has completed the study.

10.8 Study monitoring, auditing, and inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10.8.1 Safety Oversight

The Principal Investigator and Co-Investigators will be ultimately responsible for assuring the security of all study related materials to minimize risk to participants. Safety data such AEs and SAE will be assessed and reviewed in the PGx arm every 3 months after enrollment begins.

This study is expected to be classified as low risk by ACC Risk Categories for Studies. The intervention (PGx test) requires a blood sample drawn during routine laboratory blood draw (unless a saliva specimen is collected) and thus poses limited risk compared to that experienced in daily life. If officially deemed low risk by ACC, we anticipate this protocol will be monitored on a "for-cause" basis only.

10.8.2 Clinical Monitoring

There is no external sponsor for this study. The PI and study physician will periodically review the adverse events that occur during the study to determine their relatedness to the study intervention.

10.8.3 Protocol Deviations

The PI and the study team should document all scenarios where the protocol is not followed and provide, in particular:

- Who deviated from the protocol
- What was the deviation
- When did the deviation occur
- How did the deviation happen
- What is the impact of the deviation
- A root cause analysis of why the deviation occurred

Not following the genotype guided dose recommendation is NOT considered a protocol deviation, but will be recorded as one of the study outcomes.

If the assessment results in a determination that any of the following are potentially affected, the deviation would be considered of significant impact:

- having the potential to adversely affect subject safety; OR

- increases risks to participants; OR
- adversely affects the integrity of the data; OR
- violates the rights and welfare of participants, OR
- affects the subject's willingness to participate in research.
- there is a potential for an overall impact on the research that should be shared with the IRB for consideration and development of next best steps to address it

10.9 Protocol Amendment History

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by the principal investigator. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB/IEC is notified within 5 days.

Any permanent change to the protocol must be handled as a protocol amendment. The written amendment must be submitted to the IRB/IEC and the investigator must await approval before implementing the changes. The principal investigator will submit protocol amendments to the appropriate regulatory authorities.

If in the judgment of, the sponsor, the IRB/IEC, and/or the investigator, the amendment to the protocol substantially changes the study design and/or increases the potential risk to the subject and/or has an impact on the subject's involvement as a study participant, the currently approved written informed consent form will require similar modification. In such cases, informed consent will be renewed for subjects enrolled in the study before continued participation.

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale.

Version	Date	Description of Change	Brief Rationale
2.0	2-3-21	1. Addition of study personnel 2. Addition of study procedure (serum uracil collection at baseline for PCAM patients only) 3. Revision of fluoropyrimidine dose recommendations for DPYD p.Y186C variant carriers 4. Revision to PRO data outcomes tool	1. Study personnel will assist with subject recruitment and consenting. 2. Collection of serum uracil at baseline will be used as an alternative method for determining DPYD phenotype. Additional study procedure included in ICF with patient-friendly language.

Version	Date	Description of Change	Brief Rationale
			3. Dose revisions to reflect current scientific literature, clinical guidelines, and expert feedback 4. The NCI PRO-CTCAE™ tool has been revised to collect information on cardiotoxicity symptoms from subjects.
3.0	Feb 19, 2021	1.Oncology providers can obtain consent. 2. Removal of pregnancy test as study procedure 3. Collection of SSN from participants	1. Due to the busy clinic volume, the physicians wish to be able to consent patients for the study and not have to wait to study staff. 2. Pregnancy test is not performed as part of standard of care. 3. To disburse funds for participating in surveys.
4.0	Nov 10, 2021	1. Revision of specimen collection to include saliva. 2. Removal of inclusion criteria of ECOG status. 3. Removal of inclusion criteria of prior treatment of fluoropyrimidines 4. Updated timeframe for endpoint collection.	1. Provides an additional method of sample collection if phlebotomy closed for the day. 2. Patients with higher ECOG status (3 or 4) still suitable for treatment with fluoropyrimidines. Oncologist will determine suitability for treatment with fluoropyrimidines on a case by case basis. 3. Physicians have expressed interest in obtaining genotype information regardless of a patient's prior treatment status, and particularly for individuals with previous intolerance of fluoropyrimidine to guide future treatment decisions. 4. feasibility endpoint time frame revised from '7 days' to 'prior to the first dose of chemotherapy'. Seven days is less

Version	Date	Description of Change	Brief Rationale
			clinically relevant than patient-specific treatment schedules. adverse event data and PRO questionnaires to be collected over first six cycles, not months. It is not necessary to collect AE data over the longer time period for study purposes given that chemotherapy toxicity is typically experienced during initial cycles.
5.0	8.03.2023	Clarification regarding data collected at baseline and with each chemotherapy cycle.	Data elements listed in the protocol will be collected if already performed as per usual care and available in PennChart. These will not be performed for the study.
6.0	5.14.2024	Revised definition severe toxicities from ≥ Grade 3 toxicities to treatment related adverse events requiring treatment in the hospital, emergency department, or oncology evaluation center.	Prospective completion of chemotherapy related toxicity per the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) was not feasible due to a lack of a consistent research coordinator throughout the study. In addition, the completion rate for the PROs was very low therefore we could not assign a grade to the adverse event.

11 STUDY FINANCES

11.1 Funding Source

This study is financed through a grant from the Penn Center for Precision medicine.

11.2 Conflict of Interest

All University of Pennsylvania investigators will follow the University conflict of interest policy.

11.3 Participant Payments

Participants will be compensated with a $25 gift card for completing the survey on attitudes towards PGx testing (Appendix 14.7). Participants will be compensated an additional $50 for completing the microbiome sub-study.

12 PUBLICATION AND DATA SHARING PLAN

Data generated by this study will be shared at scientific meetings and published in scientific journals. Investigators actively involved in the execution of the trial will be invited to co-author publications. All study investigators will be informed in writing prior to any written communication or oral presentation about the study and invited to give comments.

Reasonable request for samples and data will be shared in a de-identified manner.

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14 APPENDIX

14.1 Schedule of Activities (SoA)

Study Procedure	Baseline/ Pre-treatment	Cycle 1	Cycles 1-6[14]	Follow-up[15]	Any point during months 1-6
	D x	D 1	D 1		
Informed consent	X				
Inclusion and exclusion criteria	X				
Demographic data[1]	X				
Cancer history[2*]	X				
Physical examination[3*]	X	X	X		
Vital signs[4*]	X	X	X		
Hematology[5*]	X	X	X		
Clinical chemistry[6*]	X	X	X		
Creatinine clearance[7*]	X	X	X		
Pharmacogenetic test[8]	X				
Blood sample for uracil measurement[9]	X				
Toxicity assessments[10*]		X	X		
Tumor outcomes[11*]				X	
Patient reported outcomes[12]	X	X	X		
Chemotherapy		X	X		
Concomitant medications[13*]	X	X	X		
Patient survey[16]					X
Stool collection[17]	X				X

***if performed as part of usual care and available in PennChart**

1. Demographic data: age, gender, race/ethnicity
2. Cancer history: GI tumor type, stage, previous cancer treatments, number of lines of therapy, ongoing toxicities related to previous therapy
3. Routine physical examination: WHO performance status, height (cm), weight (kg)
4. Vital signs: heart rate, blood pressure

5. Hematology: hemoglobin, hematocrit, white blood cell count, ANC, neutrophils, eosinophils, basophils, lymphocytes, monocytes, platelets
6. Clinical chemistry: sodium, potassium, calcium, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, total bilirubin, albumin
7. Creatinine clearance (using Cockcroft-Gault formula)
8. Pharmacogenetic test: screening for SNPs in DPYD (*DPYD**2A, *DPYD* *5, *DPYD* *6, *DPYD* *8, *DPYD* *9A, *DPYD* *10, *DPYD* *12, *DPYD* *13, rs2297595, rs115232898, rs67376798, *DPYD* HapB3 (rs75017182, rs56038477, rs56276561) and in *UGT1A1* (*UGT1A1**6, *UGT1A1**28)
9. Research blood draw to measure serum uracil (PCAM patients only)
10. Toxicity assessment: Severe treatment related adverse events requiring hospitalization, emergency department visit, or visit to the Oncology Evaluation Center (OEC) will be abstracted from medical records and adverse events reported during cycles will be documented
11. Tumor outcomes: progression free survival and overall survival.
12. Patient reported outcomes per NCI PRO-CTCAE™ and quality of life per FACT-G7 questionnaire
13. Concomitant medications: including start date, dose, unit, frequency, route of administration and indication
14. Until cycle 5, or until end of treatment because of tumor progression, unacceptable toxicity or any other reason for which treatment with fluoropyrimidines and/or irinotecan is discontinued
15. Follow-up: at 6 $\pm$ 2 months after treatment initiation
16. Electronic/paper survey on patient attitudes towards pharmacogenetics will be administered once at any point during the study once he/she has undergone pharmacogenetic testing.
17. If patients wish to participate in optional microbiome substudy (PCAM patients only)

14.2 ECOG Performance Status

Grade	ECOG Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

14.3 NCI-CTCAE version 5.0

See:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf (Control click to follow link)

The study is no longer grading adverse events using this criteria.

14.4 Medication information

14.4.1 Clinical Pharmacology of 5-FU, capecitabine and irinotecan

5-FU is an intravenous fluorine-substituted analogue of uracil that inhibits DNA synthesis to result in cytotoxicity and cell apoptosis. Capecitabine is an oral prodrug of 5-FU that is converted to 5-FU via a three-step enzymatic cascade. The amount of 5-FU available to exert its anticancer effect is directly regulated by its catabolism. Dihydropyrimidine dehydrogenase (DPD) is responsible for the initial and rate-limiting step of 5-FU catabolism. The enzyme, encoded by *DPYD*, converts approximately 80% of 5-FU in the liver into its inactive form.[25] When DPD is inactive or harbors reduced activity, the rate of 5-FU clearance decreases, leading to the development of severe fluoropyrimidine-related adverse events due to the prolonged exposure of 5-FU.

In the event of fluorouracil overdose, uridine triacetate is administered within 96 hours following the end of fluorouracil infusion.[26] Clinical presentation of acute overdose with capecitabine consists of nausea, diarrhea, vomiting, GI irritation and bleeding, and bone marrow depression. Medical management should include typical supportive medical interventions to correct the observed clinical manifestations. Dialysis may be of benefit in reducing circulating concentrations of a low-molecular-weight capecitabine metabolite (5'-DFUR), though there are no reported experiences evaluating dialysis for treating capecitabine overdose. Single doses of capecitabine were not lethal in animal studies (mice, rats, monkeys) at doses up to 2000 mg/kg (>2-9 times larger than the recommended human daily dose).[27] See Table 6 for information on the pharmacokinetics (PK) of 5-FU and capecitabine.

Irinotecan is a prodrug converted by carboxylesterase enzymes to the active metabolite SN-38 upon intravenous injection. SN-38 targets topoisomerase I to exert its cytotoxic effects by preventing DNA re-ligation of single strand breaks, establishing lethal double-stranded breaks that result in irreparable molecular damage and cell apoptosis. Due the lipophilic nature of SN-38, the metabolite undergoes phase II metabolism (glucoronidation) and becomes inactivated by uridine diphosphate-glucoronosyltransferase (UGT) encoded by the *UGT1A1* gene. Reduced enzymatic activity of UGT1A1 can lead to elevated concentrations of SN-38 and unconjugated (indirect) hyperbilirubinemia, which directly relate to the dose-limiting toxicities of febrile neutropenia and severe diarrhea.[28]

Overdose with irinotecan at doses up to approximately twice the recommended therapeutic dose have been reported, which may be fatal. The most significant adverse events were severe neutropenia and severe diarrhea. In these situations, maximum supportive care should be administered to prevent dehydration in the event of diarrhea and/or infectious complications; there is no known antidote for irinotecan overdose.[11] See Table 7 for information on the pharmacokinetics of irinotecan and its liposomal formulation.

Table 6. Pharmacokinetics of Fluoropyrimidines

PK Parameter	Fluorouracil (5-FU)[27] Following bolus injection	Capecitabine (CAPE)[27] Following 1255 mg/m^2 PO BID dose
Absorption	Not listed in package insert	**CAPE (prodrug)** / **5-FU (active metabolite)** **T_{max}** 1.5 hours / 2 hours **C_{max}** Lowered by 60% with food / Lowered by 43% with food **AUC$_{0-\infty}$** Lowered by 35% with food / Lowered by 21% with food
Distribution	Distributes throughout the body including the intestinal mucosa, liver, cerebrospinal fluid, and brain tissue.	Plasma protein binding: <60% Not concentration dependent Primarily bound to albumin (35%)
Metabolism	Remaining amount of drug following excretion is primarily metabolized in liver.	Extensively bioactivated and metabolized enzymatically to 5-FU in liver. In vitro studies indicate CAPE and its metabolites do not inhibit CYP isoenzymes 1A2, 2A6, 3A4, 2C19, 2D6, and 2E1.
Excretion Half life ($t_{1/2}$)	**Urine** 5-20% unchanged in 6 hours; metabolites over 3-4 hours **$t_{1/2}$** 8-20 min	**Urine** 95.5%; 3% unchanged **Feces** 2.6% **$t_{1/2}$** 0.75 hours (CAPE, 5-FU)

Table 7. Pharmacokinetics of Irinotecan formulations

PK Parameter	Irinotecan (IRI)[11]	Liposomal IRI[29] Following 70 mg/m^2 IV dose
Absorption	C_{max} of SN-38: within 1 hour following end of 90-min infusion of IRI See table 9 in the package insert labeling for dose-specific information from studies	**IRI** / **SN-38** **C_{max}** 37.2 mcg/mL / 5.4 ng/mL **AUC$_{0-\infty}$** 1364 h•mcg/mL / 620 h•ng/mL **CL** 0.20 L/hr / N/A
Distribution	Plasma protein binding: 30-68% (IRI), 95% (SN-38)	Volume of distribution: 4.1 L (IRI) Plasma protein binding: <0.44% (IRI)
Metabolism	Glucuronidation of SN-38 to SN-38G via UGT1A1; oxidation via CYP3A4. In vitro studies indicate IRI and its metabolites do not inhibit CYP isoenzymes.	Glucuronidation of SN-38 to SN-38G via UGT1A1; oxidation via CYP3A4

Excretion Half life ($t_{1/2}$)		**IRI**	**SN-38**	**SN-38G**		**IRI**	**SN-38**	**SN-38G**
	Urine	11-20%	<1%	3%	**Urine**	11-20%	<1%	3%
	$t_{1/2}$ (hr)	6-12	10-20	N/A	**$t_{1/2}$ (hr)**	25.8	67.8	N/A

14.4.2 Drug Interactions for 5-FU, capecitabine and irinotecan

Fluoropyrimidine Drug-Drug Interactions

There is a potential for drug interactions with fluoropyrimidines and CYP2C9 substrates and anticoagulants such as warfarin. Elevated coagulation times for warfarin have been reported with both 5-FU and capecitabine. While there is a lack of pharmacokinetic data to assess the effect of 5-FU on warfarin, altered coagulation parameters have been observed with capecitabine in addition to an increase in warfarin concentrations. These events occurred within several days and up to several months after initiation of capecitabine (and within 1 month of discontinuing capecitabine in a few cases). The maximum observed INR value increased by 91%; this increase in drug concentration is believed to be due to inhibition of CYP2C9 by 5-FU or its metabolites.

Phenytoin should also be carefully monitored and a dose reduction may be required when administering capecitabine. Toxicity associated with elevated phenytoin levels has been reported and may be due to CYP2C9 inhibition. Although no formal drug-drug interaction studies have been conducted, precautions should taken when capecitabine is coadministered with CYP2C9 substrates.

Increased concentrations and toxicity of 5-FU have been reported with leucovorin. Deaths from severe enterocolitis, diarrhea, and dehydration have been reported in elderly patients receiving weekly leucovorin and 5-FU.

Administration of infusional 5-FU does not require filter, DEHP-free, or low sorb tubing. Standard infusion tubing is used with a closed system transfer device due to the hazardous nature of the chemotherapy agent. In the event that only DEHP-free or low sorb tubing is available, these materials may still be used for dispensing 5-FU infusions, as they have not shown to be incompatible.

Fluoropyrimidine Drug-Food Interactions

Food has been shown to reduce both the rate and extent of absorption of capecitabine. In clinical trials, patients were instructed to administer capecitabine within 30 minutes after a meal. It is recommended that capecitabine be administered with food. There are no drug-food interactions listed in the package insert for 5-FU.

Irinotecan Drug-Drug Interactions

It is recommended to administer 5-FU and leucovorin prior to irinotecan given the slight reductions in C_{max} and AUC_{0-24} of SN-38 (14% and 8%, respectively) with 5-FU and leucovorin in a phase I clinical study. No formal drug interaction studies have been conducted.

It is recommended to avoid administration of strong CYP3A4 inducers with irinotecan due to substantially reduced exposure to irinotecan and SN-38 in adults and children concomitantly receiving CYP3A4 enzyme-inducing anticonvulsants (phenytoin, phenobarbital, carbamazepine) or St. John's wort. It is recommended to substitute non-enzyme inducing therapies for at least 2 weeks prior to starting irinotecan therapy (and its liposomal formulation) and to avoid strong CYP3A4 inducers unless there are no therapeutic alternatives.

Concomitant ketoconazole (CYP3A4 and UGT1A1 inhibitors) can increase exposure to irinotecan and SN-38. Coadministration of irinotecan with other strong CYP3A4 inhibitors (clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole) or other UGT1A1 inhibitors (atazanavir, gemfibrozil, indinavir) may increase systemic exposure to irinotecan and SN-38. It is recommended to discontinue strong CYP3A4 inhibitors at least 1 week prior to starting irinotecan therapy (and its liposomal formulation) and to avoid strong CYP3A4 or UGT1A1 inhibitors unless there are no therapeutic alternatives.

Administration of irinotecan does not require filter, DEHP-free, or low sorb tubing. In-line filters should not be used during the administration of liposomal irinotecan. Standard infusion tubing is used with a closed system transfer device due to the hazardous nature of the chemotherapy agent. In the event that only DEHP-free or low sorb tubing is available, these materials may still be used for dispensing irinotecan infusions, as they have not shown to be incompatible.

Irinotecan Drug-Food Interactions

There are no drug-food interactions listed in the package insert for irinotecan or its liposomal formulation.

14.4.3 Clinical Adverse Event Profile

Fluorouracil

- Increased risk of serious or fatal adverse reactions in patients with low or absent DPD activity
- Cardiotoxicity
- Hyperammonemic encephalopathy
- Neurologic toxicity
- Diarrhea
- Palmar-plantar erythrodysesthesia (hand-foot syndrome)
- Myelosuppression
- Mucositis
- Increased risk of elevated INR when administered with warfarin

Capecitabine (occurring in ≥30% patients)

- Diarrhea
- Hand-foot syndrome
- Nausea
- Vomiting
- Abdominal pain
- Fatigue/weakness
- Hyperbilirubinemia

Irinotecan (occurring in ≥30% patients receiving combination therapy)

- Nausea
- Vomiting
- Abdominal pain
- Diarrhea
- Constipation
- Anorexia
- Mucositis
- Neutropenia
- Leukopenia
- Anemia
- Thrombocytopenia
- Asthenia
- Pain
- Fever
- Infection
- Abnormal bilirubin
- Alopecia

Liposomal irinotecan (occurring in ≥20% patients)

- Diarrhea
- Fatigue/asthenia
- Vomiting
- Nausea
- Decreased appetite
- Stomatitis
- Pyrexia
- Lymphopenia (occurring in ≥10% patients with Grade 3 or 4 severity)
- Neutropenia (occurring in ≥10% patients with Grade 3 or 4 severity)

14.5 Pharmacogenetic test validation reports (to be attached)

14.6 Data collection tool for chemotherapy-related adverse events and patient reported outcomes (to be attached)

14.7 Participant survey – Pharmacogenetic testing (to be attached)

14.8 Stool collection kit instructions for optional microbiome substudy (to be attached)

14.8.1 Bristol Stool Chart for optional microbiome substudy (to be attached)

END OF DOCUMENT

IMPACT-GI Statistical Analysis Plan (SAP)

Section 1: Administrative

Section	Index	Description
Title and trial registration	1a 1b	ImpleMenting PhArmacogenetiC Testing in Gastrointestinal Cancers (IMPACT-GI); IRB #844763 (Original approval date 12/18/2020) Clinical trials.gov NCT 04736472 (registration date: January 29, 2021)
SAP version	2	SAP Version 1.0 May 2024
Protocol version	3	Protocol Version 6.0 Date
SAP revision	4	n/a
SAP contributors	5	Sony Tuteja, PharmD, Co-PI Ursina Teitelbaum, MS, Co-PI Xingmei Wang, Biostatistician
Signatures	6	Sony Tuteja Ursina Teitelbaum Xingmei Wang
Abbreviations	7	EHR electronic health record TAT turnaround time TRAE treatment related adverse event

Section 2: Introduction

Section	Index	Description
Background and introduction	7	There are known pharmacogenetic (PGx) variants in the DPYD and UGT1A1 genes associated with altered drug metabolism and prolonged drug exposure resulting in drug-related adverse events from fluoropyrimidine and irinotecan therapy. Up to 30% of patients develop severe fluoropyrimidine-related toxicity, such as diarrhea, hand-foot syndrome, mucositis, and myelosuppression at standard doses. Similarly, as many as 20-35% of patients treated with irinotecan may experience severe toxicities, ranging from diarrhea to myelosuppression. Given the life-threatening nature of severe adverse events, hospitalizations are often indicated which result in additional resource utilization costs. Subsequently, chemotherapy-related adverse events often lead to treatment delays or discontinuations that may impact tumor prognosis. However, testing for these variants is not routinely performed in clinical practice prior to the initiation of chemotherapy due to lack of a clinical assay with rapid turnaround time (TAT) and challenges in integrating genetic test results within the electronic health record (EHR). We hypothesize that providing clinicians with the ability to order rapid turnaround PGx test results along with specific dosing recommendations will increase the utilization of PGx tests to guide pharmacotherapy decisions and improve patient drug related outcomes. This is a non-randomized implementation study to determine the feasibility of establishing and integrating a PGx test into clinical care to guide chemotherapy in patients with gastrointestinal (GI) cancers. Effectiveness of the PGx-guided approach will be determined by comparing the incidence of severe treatment related adverse events to historical control group of GI cancer patients enrolled into the Penn Medicine Biobank (PMBB).
Objectives and endpoints	8	Implementation Aims: 1. To determine the <u>feasibility</u> of returning PGx results prior to the first dose of chemotherapy. 2. To determine the <u>fidelity</u> to the PGx guided dosing recommendations. 3. To determine the rate of testing among providers with patients eligible for testing Effectiveness Aims: 4. To determine if providing PGx test results will decrease the number of patients experiencing severe treatment related events during the first six cycles of chemotherapy.

		5. To determine if providing PGx test results will improve patient reported outcomes (PRO) during the first six cycles of chemotherapy.
Hypothesis	9	Providing clinicians with the ability to order a PGx test with a rapid turnaround time for results, along with specific dosing recommendations, will increase the utilization of PGx tests to guide pharmacotherapy decisions and improve patient drug related outcomes.

Section 3: Study Methods

Section	Index	Description
Trial design	10	Non-randomized, prospective, open-label implementation study to determine the feasibility of establishing and integrating a PGx test into clinical care.
Randomization	11	n/a
Sample size	12	No sample size calculation was performed for this feasibility study with pragmatic inclusion of eligible participants recruited over a 20- month period (Mar 2021- Dec 2022).
Interim analyses	13	No interim analyses planned
Timing of final analysis	14	Participants were followed until May 31, 2023, for toxicity assessments with an intention of 6-month follow-up for each participant. All analyses were performed collectively in May-June of 2024.
Timing of outcome assessments	15	Serial questionnaires assessing patient reported outcomes (PROs) for symptoms associated with to adverse events will be prospectively collected by the study team at the time of each treatment for the first 6 cycles.

Section 4: Statistical Principles

Section	Index	Description
Confidence intervals and p-value	16	No hypothesis testing was performed for the primary outcome (feasibility) or secondary implementation outcomes (fidelity, penetrance). Descriptive statistics (mean, SD, median, interquartile range, range, counts, and percentage) will be used to describe and compare (t-test or rank sum test for continuous variables and Fisher's exact test for categorical variables) baseline characteristics between the historical control group and PGx arms. For the effectiveness aims, significance was set at an alpha=0.05. No corrections were made for multiple testing. Confidence intervals pertaining to the odds of experiencing a severe TRAE were reported.
Adherence and protocol deviations	17	• Removal of inclusion criteria of ECOG status, patients with higher ECOG status (3 or 4) still suitable for treatment with fluoropyrimidines. Oncologist will determine suitability for treatment with fluoropyrimidines on a case-by-case basis. • Removal of inclusion criteria of prior treatment of fluoropyrimidines, physicians have expressed interest in obtaining genotype information regardless of a patient's prior treatment status, and particularly for individuals with previous intolerance of fluoropyrimidine to guide future treatment decisions. Participants that received the PGx test after initiation of chemotherapy, due to requests by the treating oncologist, were not included as part of the primary analysis and were reported/described separately and noted as protocol deviations. • Updated timeframe for endpoint collection, feasibility endpoint time frame revised from '7 days' to 'prior to the first dose of chemotherapy'. Seven days is less clinically relevant than patient-specific treatment schedules. adverse event data and PRO questionnaires to be collected over first six cycles, not months. It is not necessary to collect AE data over the longer time period for study purposes given that chemotherapy toxicity is typically experienced during initial cycles. • Prospective completion of chemotherapy related toxicity per the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) was not feasible due to a lack of a consistent research coordinator throughout the study. In addition, the completion rate for the PROs was very low (22%) therefore we could not assign a grade to the adverse event. Revised definition severe toxicities from ≥ Grade 3 toxicities to treatment related adverse events requiring treatment in the hospital, emergency department, or oncology evaluation center.

Analysis population	18	Modified intent to treat, removing participants that did not meet the inclusion criteria (i.e., received PGx test after chemotherapy or did not receive qualifying chemotherapy agent).

Section 5: Trial population

Section	Index	Description
Screening data	21	Clinic schedules will be screened by the clinical research coordinator for new patients with GI cancer being evaluated for treatment. Screen failures in this study will be defined as participants who meet criteria for study enrollment but decline to participate or the treating oncologist declines participation of their patient in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, and eligibility criteria.
Eligibility	22	Inclusion criteria: 1. Able and willing to provide informed consent 2. Pathologically confirmed gastrointestinal malignancy for which treatment with a fluoropyrimidine and/or irinotecan is indicated 3. Willing to undergo blood or saliva sampling for PGx testing and comply with all study-related procedures 4. Life expectancy of at least 6 months Exclusion criteria: 1. Prior treatment with irinotecan 2. *DPYD* or *UGT1A1* genotype already known 3. Severe renal or hepatic impairment (or unacceptable laboratory values), including: • Neutrophil count of <1.5 x 10^9/L, platelet count of <100 x 10^9/L • Hepatic function as defined by serum bilirubin >1.5 x upper limit of normal (ULN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) >2.5 x ULN, or in case of liver metastases ALT and AST>5 x ULN • Renal function as defined by serum creatinine >1.5 x ULN, or creatinine clearance <60 ml/min (by Cockcroft-Gault Equation) 4. Women who are pregnant or breast feeding, or subjects who refuse to use reliable contraceptive methods throughout the study 5. Treating physician does not want subject to participate

Recruitment	23	Prior to the scheduled visit, the research coordinator will notify the treating oncologist about potential eligible participants and remind them of the study. The treating oncologist will discuss the study with the patient during the evaluation visit and consent will be obtained by the treating GI oncology provider (physician or advanced practice provider) or the research coordinator. This will occur in person or by remote consent. Patients will be given a copy of the official informed consent form and an opportunity to ask questions. Patients will be given sufficient time to consider participating in the trial.
Withdrawal/follow-up	24	Participants are free to withdraw from participation in the study at any time upon request, without prejudice to their medical care, and are not obliged to state their reasons. The study investigator may discontinue or withdraw a patient from the study at any time for the following reasons: pregnancy, patient transfers care outside of Penn Medicine, treating oncologist wishes patient to withdraw. The reason for participant discontinuation or withdrawal from the study will be recorded on the patient's Case Report Form (CRF). Subjects who sign the informed consent form but do not undergo genotyping may be replaced. A participant will be considered lost to follow-up if he or she fails to return for completion of scheduled chemotherapy infusion appointments. There are no study specific visits. Data is collected at the time of usual clinic visit and from the medical record. The research coordinator will contact the treating oncologist and ensure the reason for not returning is not a (serious) adverse event ((S)AE). If treatment is prematurely discontinued, the primary reasons for discontinuation must be recorded in the patient's file and all efforts will be made to complete and report the observations as thoroughly as possible. Information will be extracted from the medical records and/or tumor registry at the 6-month (+ 2 months) follow-up period if performed as part of usual care and recorded in the medical record.
Baseline patient characteristics	25	The following information will be obtained at screening and from the patient medical record if performed as part of usual care and available in the medical record: • Signed informed consent form • Inclusion and exclusion criteria • Demographic data: age, gender, race/ethnicity • Cancer history: GI tumor type, stage, previous cancer treatments, number of lines of therapy • Routine physical examination: ECOG performance status (see Appendix 14.2), height (cm), weight (kg) • Vital signs: heart rate, blood pressure

		• Hematology: hemoglobin, hematocrit, white blood cell count, ANC, neutrophils, eosinophils, basophils, lymphocytes, monocytes, platelets • Clinical chemistry: sodium, potassium, calcium, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, total bilirubin, albumin • Creatinine clearance (using Cockcroft-Gault formula) • Concomitant medications (including dose, unit, frequency, route of administration and indication)

Section 6: Analysis

Section	Index	Description
Outcome definitions	26	1. Primary outcome: Feasibility: the number and proportion of tests returned prior to the first dose of chemotherapy. 2. Secondary outcomes: a. Fidelity – proportion of dose proportion of dose modifications made in agreement with the genotype guided dosing recommendations at the first dose. b. Penetrance- proportion of tests orders compared with the number of patients treated with study chemotherapy. c. Treatment-related adverse events - proportion of patients experiencing a serious chemotherapy related toxicity defined as requiring hospitalization, visit to the emergency department or oncology evaluation center (OEC). d. Relative dose intensity - cumulative dose administered divided by the anticipated cumulative dose over six cycles reported as mg/m2. e. Patient reported outcomes (PROs) during the first six cycles of chemotherapy. f. Patient knowledge and attitudes towards PGx testing.
Analysis methods	27	1. Primary outcome: We will report the number and proportion of tests returned prior to the first dose of chemotherapy in the prospective testing arm only as this outcome was not relevant for the historical control group. The proportions were compared among clinical sites using a chi-square test. The test turn-around time was reported as median (interquartile range) and compared using the Wilcoxon rank-sum test. 2. Secondary outcomes: a. Fidelity- proportion of dose modifications made in agreement with the genotype guided dosing recommendations at the first dose was reported, no hypothesis testing was performed. b. Penetrance- proportion of tests orders divided by the number of patients eligible for testing based on pharmacy records indicating the number of fluoropyrimidine prescriptions during the enrollment time frame, no hypothesis testing was performed. c. Treatment-related adverse events (TRAE): proportion of patients experiencing a severe treatment related adverse event defined as those requiring hospitalization, visit to the emergency department, or visit to the oncology evaluation center (OEC). The proportion of patients with severe TRAEs in the prospective cohort were compared between the DPYD variant carriers vs DPYD wild-type patients. The proportion of patients with TRAEs were compared between the prospective and historical control groups among DPYD variant carriers. The proportion of patients with TRAEs in the prospective cohort were compared between the UGT1A1 poor metabolizers vs UGT1A1 normal and intermediate metabolizers. The

		proportion of patients with TRAEs were compared between the prospective and historical control groups among UGT1A1 poor metabolizers. Chi-square tests were used for all comparisons. Factors contributing to having a TRAE were evaluated using a multivariable logistic regression model adjusting for age, sex, race, tumor type, ECOG score, presence of metastases, intent of treatment, DPYD phenotype, and UGT1A1 phenotype using a stepwise approach. d. Relative dose intensity for fluoropyrimidines and irinotecan, defined as cumulative dose administered divided by the anticipated cumulative dose over six cycles reported as mg/m2 was compared between variant carriers and wild-type patients (or PM vs. IM/NM for irinotecan) in the prospective cohort using t-tests. Relative dose intensity for fluoropyrimidines and irinotecan, was compared between the prospective and historical control groups in variant carriers (or PM for irinotecan) using t-tests. e. Patient reported outcomes (PROs) during the first six cycles of chemotherapy were compared in the prospective cohort between DPYD variant carriers and wild-type patients using chi-square tests in the subset that completed PROs. f. Patient knowledge and attitudes towards PGx testing were evaluated using chi-square and t-tests as appropriate.
Missing data	28	a. Prospective completion of chemotherapy related toxicity per the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) was not feasible due to a lack of a consistent research coordinator throughout the study. In addition, the completion rate for the PROs was very low therefore we could not assign a grade to the adverse event. b. Revised definition severe toxicities from ≥ Grade 3 toxicities to treatment related adverse events requiring treatment in the hospital, emergency department, or oncology evaluation center.
Additional analyses	29	a. The number of treatment modifications (i.e., delay of treatment or dose reduction) and treatment discontinuations in the prospective cohort were compared between the DPYD variant carriers vs DPYD wild-type patients using chi-square tests. These outcomes were also compared between the prospective and historical control groups among DPYD variant carriers. These analyses were also performed for the UGT1A1 phenotype. b. Overall survival at one year was reported for the prospective and historical cohorts.
Harms	30	No harms of the genotyping intervention were reported.
Statistical software	31	SAS version 9.4 and R version 4.2.3
References	32	1. Henricks LM, Lunenburg CATC, De Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *The Lancet Oncology*. 2018;19(11):1459-1467. doi:10.1016/S1470-2045(18)30686-7 2. Amstutz U, Henricks LM, Offer SM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine

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