Implementing Genomics in Practice (IGNITE) Proof of Concept Study: *CYP2C19* genotype-supported treatment of GERD and dyspepsia versus conventional treatment

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UF Protocol and Statistical Analysis Plan

1. Project Title:

Implementing Genomics in Practice (IGNITE) Proof of Concept Study: *CYP2C19* genotype-supported treatment of GERD and dyspepsia versus conventional treatment

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3. Abstract:

Proton Pump Inhibitors (PPIs) are indicated for the treatment of gastroesophageal reflux disease (GERD), *H. pylori* infection, peptic ulcer disease, and gastric protection in patients at risk for gastrointestinal bleeding. PPIs are among the most commonly prescribed drugs in the world, and their most common indication is GERD. Refractory GERD is the term used to describe non-responsiveness to conventional PPI therapy, and it occurs in up to 40% of patients even when the PPI dose is doubled. The efficacy of PPIs is highly dependent on plasma concentrations achieved following drug administration. All PPIs are metabolized in part by the CYP2C19 enzyme, which is encoded by the highly polymorphic *CYP2C19* gene. Depending on the *CYP2C19* genotype, individuals are classified into different metabolizer phenotypes: poor metabolizers (PM, 2 loss-of-function *CYP2C19* alleles); intermediate metabolizers (IM, one loss-of-function allele); normal metabolizers (NM, no loss or gain-of-function alleles); rapid metabolizer (RM; one gain-of-function allele) and ultra-rapid metabolizers (UM, two gain-of function-alleles). Genetic variants in *CYP2C19* are known to profoundly influence PPI plasma concentrations and consequently, response to PPI therapy. For example, individuals classified as either RM or UM have lower PPI concentrations compared to NM, IMs, or PMs, respond poorly to PPI therapy, and some fail to respond even when the PPI dose is increased. **We hypothesize that genotype-supported treatment of GERD and dyspepsia will lead to better GERD control and improvement in severity of dyspepsia symptoms compared to conventional treatment.** We will conduct a comparative effectiveness study of genotype-supported vs. conventional treatment of GERD and dyspepsia. Patients presenting with GERD or dyspepsia symptoms and either 1) being initiated on PPI therapy or 2) with continued symptoms on current PPI therapy will be recruited from gastroenterology clinics and randomized to a genotype-supported versus conventional PPI therapy management strategy. We will integrate individual *CYP2C19* genotype information into treatment decisions for the genotype-supported arm and compare change in symptom control from baseline to the end of the study between study arms. Given that PPI efficacy is related to PPI exposure and to metabolizer phenotype, individualizing treatment using *CYP2C19* genotype information is expected to improve symptom

management. We will also evaluate patient and clinician knowledge, attitudes, and beliefs about pharmacogenetic testing and physician acceptance of genetic information into clinical practice. Finally, we will collect preliminary data on the potential impact of *CYP2C19*-supported PPI dosing on adverse event rates.

4. Background:

In the past 10 years, there have been significant advances in defining genetic determinants of drug response. These advances have led to the expectation by many that eventually an individual's personal genetic information will become part of his or her medical record to be used to guide treatment decisions. The UF Health Personalized Medicine Program (PMP) was created at UF to meet this expectation. The initial efforts of the PMP involved implementation of cytochrome P450 (CYP) 2C19 genotyping to guide antiplatelet therapy following percutaneous coronary intervention.

At UF Health, *CYP2C19* genotyping is available clinically. However, the gastroenterologists do not use *CYP2C19* genotype information, and there is a need for further evidence generation, we which propose to do through a pilot implementation study. The proposed study is significant because it may extend the evidence regarding influence of *CYP2C19* genotype in PPI efficacy, lead to more rationale dosing of PPIs and management of patients with GERD or dyspepsia, and build a body of evidence to facilitate the implementation of pharmacogenetic-supported GERD and dyspepsia management.

GERD Refractory to PPI Treatment. Gastroesophageal reflux disease (GERD) affects 10-20% of individuals in Western countries[1,2]. PPIs are agents of choice to treat GERD, and are among the highest-selling classes of drugs in the US, with $9.5 billion in sales in 2015. Six PPIs are commercially available: omeprazole (Prilosec); lansoprazole (Prevacid), dexlansoprazole (Dexilant) esomeprazole (Nexium), pantoprazole (Protonix), and rabeprazole (Aciphex). Low doses of omeprazole and lansoprazole can be purchased over the counter. While effective for symptom relief and erosive esophagitis, PPI treatment fails in up to 40% of patients with GERD[11]. Failure to respond to standard, once daily PPI treatment or complete or partial response to twice daily PPI treatment has been defined as refractory GERD[1,12]. Several factors have been associated with refractory GERD, including adherence to PPI therapy, esophageal hypersensitivity and inter-individual differences in PPI metabolism[11].

CYP2C19 genetic variation influences the pharmacokinetics of PPIs. PPIs inhibit the final pathway of acid production in gastric parietal cells by covalently binding to the H+/K+-ATPase proton pump leading to inhibition of acid secretion, which lasts for 24-48 hours despite the drug's short pharmacokinetic half-life[13-15]. Most PPIs are metabolized by CYP2C19 and CYP3A4; have short elimination half-lives (1-4 hours) and distribution volumes that are 8%-20% of total body water. All PPIs have low hepatic clearances, low first-past metabolism and have high bioavailabilities[6]. The *CYP2C19* gene that encodes the CYP2C19 enzyme is located on chromosome 10q24.1-q24.3, has 9 exons and is highly polymorphic[16-18].

Several loss of function alleles (e.g., *CYP2C19**2 through **9*) reduce drug clearance and significantly increase PPI plasma concentrations and area under the plasma concentration-time curve (AUC). Carriers of one or two loss of function alleles are referred to as intermediate and poor metabolizers (IMs and PMs), respectively. The extent to which

CYP2C19 variation affects clearance depends on the fraction of total clearance attributable to CYP2C19. All PPIs undergo significant metabolism by CYP2C19, and genetic variation in *CYP2C19* influences the pharmacokinetics of all PPIs.[6,19,20]. The G681A polymorphism causes a splicing defect in exon 5 resulting in protein synthesis termination and is responsible for the **2* allele, the most prevalent LOF allele. The wild type (WT) *CYP2C19*1* allele is the nomenclature assigned when no functional variant alleles are present, and is associated with a more rapid clearance and lower AUCs compared to the PM or IM phenotypes. Those who are homozygous for **1* are referred to as and are responsible for normal metabolizers (NM)[17]. *CYP2C19*17* is a gain- of-function allele that increases the clearance of PPIs and reduces AUC compared to NMs. The **1/*17* or **17/*17* genotypes contribute to the rapid or ultra-rapid metabolizer phenotype (RM or UM, respectively)[21,22]. The AUC following the administration of equal PPI doses can vary 3-10 fold between PMs and UMs.[6,23-26] Table 1 shows population frequency data for the various genotypes/inferred phenotypes from the UF clinical implementation of *CYP2C19* genotyping for clopidogrel response prediction.

TABLE 1. CYP2C19 genotypes, inferred phenotypes, and population frequencies

ALLELE[a]	GENOTYPE/ACTIVITY	PHENOTYPE[b]	FREQUENCY
**1/*1*	Wild Type (WT)/ 2 active alleles	NM	39%
**1/*n*	Heterozygous WT/1 active, 1 LOF allele	IM	20%
**2/*2 or *n/*n*	Homozygous variant/2 LOF alleles	PM	1.7%
**1/*17*	Heterozygous WT/1 active, 1 GOF allele	RM	28%
**17/*17*	Homozygous variant/2 GOF alleles	UM	5.3%
**2/*17*	Heterozygous; 1 LOF/ 1 GOF allele	?	5.6%

[a] **2, *3, *8,* or **9* refer to loss-of-function (LOF) alleles, n refers to any of the LOF alleles

[b] Abbreviations: GOF –gain of function; NM—normal metabolizer; IM – intermediate metabolizer; PM – poor metabolizer; RM—rapid metabolizer; UM—ultra-rapid metabolizer

Efficacy of PPIs depends on plasma concentrations and *CYP2C19* genetic variation. Studies report that the efficacy of PPIs to treat GERD and related conditions is closely linked to plasma concentrations[27-30]. Numerous studies in adults have shown that *CYP2C19* variants markedly influence the pharmacokinetics and pharmacodynamics of PPIs (see reviews[6,16-18,23,31-33]). *H pylori* cure rates are significantly higher among PMs compared to RMs,[24,25] and higher doses of PPI are required to treat *H pylori* in RMs[25,34].

Long-term PPI use is associated with increased risk of adverse events. Use of PPIs has increased dramatically in the last decade, driven in part by an impression that they are "benign drugs". As a result they are often prescribed as long term therapy in multiple different patient populations. It is not uncommon for patients to be on PPI therapy for multiple years or even "indefinitely", despite no clear indication being present. However, there is mounting evidence that long-term use of PPIs are associated with a number of significant adverse effects including gastric and respiratory infections, bone fractures and kidney disease[10,37]. For example, studies have reported increased risk of intestinal infections with *Salmonella, Clostridium difficile, Shigella, Campylobacter, Streptococcus, Lactobacilli* and fungus with PPI use (see reviews[40-46]). This is believed to be due to reduced effectiveness of the pH barrier in the stomach to protect against infections from ingested bacteria and viruses with chronic gastric acid suppression[38,39]. If aspirated during reflux episodes, infected gastric media may increase the risk of upper respiratory infections[48,49]. Several studies have reported associations between the chronic use of PPIs and upper respiratory infections and community-acquired pneumonia[40,52-60]. PPI use has also been associated with bone fractures in young adults[68]. Recent studies have also

linked PPI use with the risk of chronic kidney disease (CKD)[73,74] and with progression to end-stage renal failure (ESRD)[75,76]. Additionally, there is evidence suggesting that higher daily PPI doses and/or the degree of acid suppression increase risk for PPI-associated adverse effects,[47,58,74] thereby supporting a role for *CYP2C19* genetic variation contributing to adverse events. While the primary focus of this proposal is the impact of *CYP2C19* genotype-supported management of GERD and dyspepsia, we will also collect follow-up data on the occurrence of gastrointestinal and pulmonary/upper respiratory infections, bone fracture, and adverse kidney outcomes to begin building evidence on whether genotype-supported treatment of GERD and dyspepsia may influence risk for adverse outcomes.

This study is significant because it will prospectively test the hypothesis that genotype-supported treatment in patients with GERD and/or dyspepsia leads to better symptom control compared to conventional treatment. Data on adverse events with *CYP2C19*-supported treatment gained from this study will be used to inform power calculations for a larger study focused on preventing PPI-associated adverse events through genotype-supported dosing.

Table 2. Comparison of lansoprazole plasma concentrations by CYP2C19 phenotype.

Phenotype	Alleles	Number Participants	Mean ±LZ Con. ng/ml
NM	*1/*1	11	199±137
IM	*1/*2 or *3	13	303±165
RM	*1/*17	11	168±146
UM	*17/*17	2	156±123
?	*2/*17	4	201±57.1

A companion study in children will be conducted at Nemours Children's Hospital Gastroenterology Clinics in Orlando. The preliminary data below were generated by investigators at Nemours and helped to inform sample size calculations for our study.

5. Preliminary Data

Lansoprazole pharmacokinetic data and *CYP2C19* genotype. Table 2 demonstrates the relationship between plasma drug concentrations (AUC) and *CYP2C19* genotype.

The data in Table 2 were collected from children with asthma taking a 30-mg lansoprazole (LZ) dose daily for 6 months[61,63]. Blood was drawn 2 hours after the final dose, which corresponds to time at which blood levels peak after an oral dose. Note that compared to individuals with the NM phenotype, LZ concentrations were higher in carriers of one loss-of-function allele (**1/*2* or **3*; IM) indicating decreased clearance. Carriers of **17* gain-of-function allele (RM) had lower LZ concentrations compared to NMs or PMs, indicating higher clearance. Though the number of participants is small, **17/*17* participants (UMs) had the lowest LZ concentrations; and for **2/*17* participants levels were similar to **1/*1* alleles (NM phenotype). These preliminary data support the hypothesis that *CYP2C19* variations influence the PK of LZ in children and that *CYP2C19* genotype can be used to discriminate metabolizer phenotypes.

Association Between CYP2C19 Phenotype and pH Testing Outcomes in Children taking PPIs. Esophageal pH probe testing is commonly performed in children to assess the efficacy of PPI medication therapy for GERD. We hypothesized that the *CYP2C19* gain-of-function genotype among children who have undergone esophageal pH probe testing while

on PPI therapy would be associated with pH probe acid exposure outcomes. Tissue collected during pH probe testing was genotyped in 74 children using Taq Man techniques. Children with the RM phenotype (gain-of-function genotype) had a poorer response to PPI (e.g. had more time with pH <4) compared to PM and NM phenotypes (Table 3). Our data support that *CYP2C19* variants influence response to PPI and suggest that genotype-supported PPI dosing may avoid pH probe testing in some children. We also hypothesize the same would be observed in adults.

Comparison of *CYP2C19* SNP Frequencies in Controls vs. Children Undergoing Fundoplication.

Table 4 shows *CYP2C19* *2 (loss-of-function) and **17* (gain-of-function) allele frequencies in children who were on PPIs and underwent fundoplication surgery compared to controls (consisting of asthmatic patients who participated in the PPI trial)[61]. DNA for genotyping was obtained from saliva samples collected from controls, and for cases was obtained from tissue samples collected from children during fundoplication surgery. The frequency of the minor **2* allele (A allele in Table 4) (PM or IM phenotype) was under-represented in the fundoplication cohort compared to controls, and the minor allele for the **17* allele (T allele) (RM phenotype) was over-represented in the fundoplication cohort compared to controls. These data suggest that RMs are more likely to be undertreated with usual doses of PPI therapy and as a result are subjected to invasive management approaches. In contrast those with the PM phenotype are effectively treated with a usual dose and so are under-represented in the surgical group. These data could be inferred to suggest that genotype-supported PPI dosing could result in dose increases in RMs, which may have the potential to reduce the number of children who would need to undergo fundoplication surgery.

Table 3. CYP2C19 phenotype and PPI efficacy

CHARACTERISTIC	GOF (N=21)*	LOF (N=53)	RR (CI)	P value
Caucasian, %	76	71		NS
Male, %	66	62		NS
Mean Age at pH test, years(SD)	8(4.6)	8(4.9)		NS
Mean PPI Dose(SD), mg/kg	1.26(0.45)	1.00(0.62)		NS
% time pH < 4	5.71	2.67	1.6 (1.1-2.3)	<0.005
Mean Acid clearance (SD)	181(271)	107(158)	2.2 (1.5-3.2)	<0.0001
Mean Number Acid Reflux Episodes(SD) in 24 hrs	25(35)	24(32)	0.71-1.5	NS

*GOF = Gain-of-function--*17 carriers, LOF = Loss-of-function carriers + no variant alleles

Table 4. CYP2C19 genotype frequencies in patients undergoing fundoplication and PPI-treated controls.

SNP/GENOTYPE	CONTROL[1]	FUNDOPLICATION
*2 (rs4244285)	Number (Percent)	Number (Percent)
GG	199 (71.5%)	31 (91.2%)
GA	75 (27.0%)	3 (8.8%)
AA	4 (0.15%)	
MAF	**0.149**	**0.044**
p value	**0.010**	
*17 (rs12248560)		
CC	178 (64.0%)	17 (50%)
CT	91 (32.6%)	14 (41%)
TT	10 (3.50%)	3 (9.0%)
MAF	**0.200**	**0.294**
p value	**0.033**	

Collectively, these preliminary data provide compelling evidence for the potential clinical utility of *CYP2C19*-genotype supported dosing by documenting 1) the relationship between *CYP2C19* genotype and PPI pharmacokinetics, 2) that response to PPI therapy, based on gastric pH, is influenced by *CYP2C19* genotype, and 3) that patients are undergoing risky, invasive procedures who may simply need a higher dose of PPI to overcome their RM phenotype status.

6. Specific Aim:

Implement pharmacogenetic testing of the *CYP2C19* gene-PPI drug pair in the context of a comparative effectiveness genotype-supported vs. conventional treatment trial. Given that PPI efficacy is related to PPI exposure and to metabolizer phenotype, individualizing treatment using *CYP2C19* genotype-supported dosing is expected to improve symptom control. **We hypothesize that genotype-supported treatment of GERD and dyspepsia will lead to better GERD control and improvement in the severity of dyspepsia symptoms compared to conventional treatment.** We will also survey patients and clinic staff on knowledge, attitudes, and beliefs toward pharmacogenetic testing, evaluate physician acceptance of genetic information into clinical practice, and collect preliminary data from the electronic health records on the potential impact of *CYP2C19*-supported treatment on the adverse event rate of upper respiratory infection (cold), sore throat, strep throat, bronchitis, pneumonia, ear infection, acute sinusitis (sinus infection), bone fractures, renal function, and gastrointestinal infections.

7. Research Plan:

The study is a randomized, open-label, comparative effectiveness clinical trial in 180 total patients with GERD or dyspepsia symptoms who will be randomized to either conventional or genotype-supported treatment of GERD and dyspepsia. A total of 120 patients will be enrolled in the UF Health Gastroenterology Clinics in Gainesville, and 60 will be enrolled in a parallel study (under separate IRB protocol) at Nemours Children's Hospital Gastroenterology Clinics in Orlando, FL. Each site will be responsible for overseeing patient care and research at their respective facility. All participants will have symptoms of GERD or related diseases and be candidates for PPI therapy or on PPI therapy with continued symptoms.

We will enroll a total of 180 patients between the UF Health and Nemours sites, which should allow for sufficient power to detect our efficacy endpoint (see power calculation below). We will also collect data from the health records for up to 24 months after enrollment on upper respiratory infection (cold), sore throat, strep throat, bronchitis, pneumonia, ear infection, acute sinusitis (sinus infection), bone fractures, renal function, and gastrointestinal infections for preliminary assessment of the effects of *CYP2C19*-supported PPI dosing on adverse effects. This exploratory arm will provide data to inform power calculations for a larger study focused on preventing PPI-associated adverse events through genotype-supported dosing.

Inclusion Criteria. A total of 120 patients with GERD or related symptoms will be enrolled at UF Health. An additional 60 patients will be enrolled at Nemours Children's Hospital Gastroenterology Clinics under a separate IRB protocol. For this protocol, any patient over the age of 17 who presents to GI clinic with GERD or related symptoms and is either 1) being initiated on PPI therapy or 2) continues to have symptoms despite PPI therapy will be considered eligible for the study.

Exclusion Criteria. This study will exclude patients who have had extensive esophageal or gastric surgery or any major chronic illness or condition that in the opinion of the gastroenterologist that would interfere with participation in the study.

Participant Recruitment Methods.

Prior to enrolling their patients, clinicians at participating GI clinics (investigators for this study) will be asked to complete surveys that assess their knowledge, attitudes, and beliefs as they relate to pharmacogenetics testing.

Patients will be approached for participation at their GI clinic visit by the treating clinician and or physician assistant. After providing informed consent, patients will be randomly assigned to either conventional treatment or genotype-supported PPI therapy using a randomization table. A 5-ml (one teaspoon) venous blood sample will then be drawn by a nurse, physician assistant, or phlebotomist. The patient will be asked to complete 3 surveys/questionnaires: the Reflux Disease Questionnaire (RDQ), Global Overall Symptom (GOS) scale, and a survey to assess knowledge, attitudes, and beliefs about pharmacogenetic testing. Patients will receive a total of $25 for participating in this study. The $25 VISA gift card will be provided after completing the final questionnaire. Patients in the control group may be withdrawn prior to completion if they or their physician elect to order the genetic test during the study. These patients would receive a $10 VISA gift card for their participation up to the point of withdrawal.

Genotyping and result reporting. Genotyping will be done by the CLIA-licensed UF Pathology Laboratory, as has been done for over 1,600 patients in the UF Health *CYP2C19*-clopidogrel implementation, with genotype results reported in the EHR. For patients randomized to the genotype-supported arm, genotype results will be available in approximately 2 weeks. For patients randomized to the conventional treatment arm, the genotype results will be resulted in the EHR after the patient completes study participation (12 weeks after patient enrollment). The patient's clinician will be educated about the genotype results and their implication for PPI response. A recommendation for a dose increase will be provided for RMs and UMs (approximately 30% to 35% of patients). For patients with other genotypes, clinicians will be advised that usual PPI doses are expected to be effective that lower doses may be sufficient for symptoms management in PMs. Clinicians will also be provided quick reference guides that will include phenotype, genotype, dosing recommendations, and educational information[81]. The PMP Committee has convened with the GI clinicians to evaluate and approve the dosing recommendations for adults based on *CYP2C19* genotype. Clinical decision support tools in the EMR will also be implemented that will provide this guidance to the treating clinician. While dosing recommendations will be provided based on genotype results, the ultimate prescribing decision will be left to the discretion of the treating physician.

Assessment of PPI efficacy and adverse effects. Our primary outcome will be the effectiveness of PPI therapy, which will be determined via two questionnaires: RDQ and GOS scale, administered by study personnel at baseline and 12 weeks. The RDQ was developed to monitor treatment response over time and evaluates 6 symptoms (12 items) covering 3 domains: heartburn, regurgitation, and upper abdominal pain. Each symptom is evaluated using a 6-point Likert scale to assess frequency and severity over the previous week. Each symptom is rated from 1 (did not have) to 6 (severe), and the RDQ mean score is calculated as the mean response to the 12 items. The RDQ mean score thus ranges from 1 to 6 and has been psychometrically validated[83]. The GOS scale evaluates 18 symptoms, with each rated from 1 (no problem) to 7 (very severe). Similar to the RDQ, the mean score is calculated as the mean response to the 18 items, with mean scores ranging from 1 to 7. Patient participation will end after they complete the approximate 12 week questionnaire. However, we will continue to

follow patients over 24 months through medical record review to assess adverse outcomes for occurrence of upper respiratory infection (cold), sore throat, strep throat, bronchitis, pneumonia, ear infection, acute sinusitis (sinus infection), bone fractures, renal function and gastrointestinal infections.

Metrics for assessing success of pharmacogenetic testing in clinical practice. We will test a variety of variables that will provide insight into attitudes and acceptability of pharmacogenetic testing. These will include: 1) percent of patients approached about the study who agree to participate; 2) surveys of patients and clinic staff on knowledge, attitudes, and beliefs about pharmacogenetic testing; and 3) the percent of the time a dosing recommendation for a patient in the genotype-supported arm is accepted by the clinician. Patients will be surveyed about knowledge, attitudes, and beliefs about pharmacogenetic testing using questionnaires on enrollment and at the end of their study participation. Clinic staff will be surveyed about knowledge, attitudes, and beliefs about testing at the beginning and end of the study.

Feasibility:

The study is feasible for enrollment over 9 months, with 3 months follow-up for efficacy based on volume of the clinics at UF. Specifically, the three adult GI providers at UF Health who will be participating in this trial will enroll patients from 10 clinics/wk, which represents approximately 150 patient visits/wk. Of these, approximately 40% of patients are being seen for peptic acid/GERD indications which should produce 50-60 pts/week who meet inclusion criteria. Assuming only half of eligible patients consent to the study, we anticipate enrolling the 120 adults within a 12 week period.

Analysis Plan and Power Calculations:

We will test the hypothesis that RMs/UMs (1 or 2 gain-of-function alleles) in the genotype-supported arm have better GERD and symptom control than RMs/UMs in the conventional treatment group because they will have their dose increased based on their genotype.

Continuous variables will be compared between the genotype-supported group and the conventional treatment group using the Student's unpaired *t*- test, and categorical variables will be compared using the chi-square or Fisher's exact test. The hypotheses will be tested using linear regression analysis to estimate the difference in the RDQ reduction in the RM/UM patients between the two arms. All analysis will be performed in SAS 9.4 (Cary, NC).

We will evaluate the percentage of the time when a dosing recommendation was made and accepted by the treating clinician, to provide practical data on the willingness of clinicians to adopt this approach. We will also collect patient- and provider-level survey data on knowledge, attitudes, and beliefs about pharmacogenetics testing, using survey tools adapted from other IRB-approved studies.

Power calculation. While there are no data in the literature on genotype associations with the efficacy endpoint we will use (symptom improvement), there are pH probe data by genotype that were generated by the Nemours investigators, that we can use, and this should be an excellent surrogate for symptom data. We have done power calculations based on two of the pH-based response phenotypes, found in Table 3. Specifically we powered based on percent time with pH < 4 (which has an effect size of 0.79) and acid clearance time (effect size of

0.45). Based on these two phenotypes, we have the following power to detect differences in efficacy between *CYP2C19*17* carriers and non-carriers:

Based on the previous data for acid clearance time, with 180 total participants (120 from UF Health and 60 from Nemours), we have 81% power to detect a 0.49 effect size.

Based on the previous data for time with pH <4.0, with 180 participants, we have >99% power to detect a 0.79 effect size.

With 180 total patients (90 patients in each arm), we expect approximately 30 patients in each are to have the genotype of interest (i.e. RM or UM). At alpha of 0.05, we will have 80% power to detect effect size of ≥0.74 in the RDQ score in RMs/UMs. This effect size is equivalent to a RDQ score difference of 1.22 for regurgitation and a score difference of 0.93 for heart burn. These differences in RDQ scores are smaller than what is considered clinically significant. Therefore our study is well-powered[84].

In summary, by studying a total 180 patients (120 from UF Health and 60 from Nemours), the data suggest we will have excellent power to detect an efficacy endpoint. Data generated from this protocol on adverse effects will also help us define the incidence of the adverse effect phenotypes to inform sample size for a future pragmatic trial examining effects of genotype-supported dosing on PPI safety endpoints.

8. Resource Sharing Plans

All educational materials and questionnaires developed for this study (including REDCap templates) will be made available to the larger UF and scientific community. Genotype data will be available in EPIC, and thus can be accessed (deidentified) through the Integrated Data Repository (IDR) for future studies. As part of the IGNITE network, we are asked to share de-identified datasets with the other IGNITE network site investigators, primarily the IGNITE coordinating center, to be used for network wide analysis. Deidentified datasets may also be shared between UF and Nemours for cross site analysis for this project. We will include a section in the consent form that allows patients to choose whether or not to share de-identified genotype data with the National Institutes of Health using the dbGaP database. We will share PGX data and core phenotypes of interest with the larger scientific community through dbGaP if the parents and patients give permission for this type of data sharing.

9. Data Safety Monitoring Plan

Based on assessments of the risk-to benefit ratio, the risk level associated with the study is low. All risks have been described in the consent form.

Monitoring for documentation of an adverse event, whether anticipated or unanticipated, is the responsibility of the principal investigator who will maintain oversight but may delegate collection of information related to this function to other study team members. In term of SAFETY monitoring, all adverse events spontaneously reported, elicited, or observed by the investigators will be recorded. All Serious Adverse Events, should they occur, whether study-related or expected, will be documented on a Case Report Form, under Adverse Event section in the Patient's binder, reported by the principal investigator to the IRB within five (5) working days.

In addition, the principal investigator will follow the reporting requirements for serious and unexpected adverse events outlined in the UF IRB Adverse Event Evaluation and Reporting Guide. All unanticipated, serious, fatal and/or life-threatening adverse events will be reported to

the UF IRB. Aggregate reports of adverse events will be prepared on an annual basis or at the end of the study, whichever may occur earlier and forwarded to the IRB at annual review.

Plan for data management: Case Report Form (CRF) within REDCap will be used for each subject. To protect the participant's right of privacy, subjects' individual records related to the study and will be stored in locked cabinets with limited access, and electronic files will be kept in secured database. A de-identified dataset from the database using a patient identification number will then be shared with statistician for analysis. During the study, data will be analyzed as it becomes available by Dr. Lari Cavallari and the investigator steam. No early closure is planned because of the limited scope and projected low risk of the study.

There is a prospect of direct benefit to individual subjects, in that genotype information could lead to improved patient outcomes.

Nevertheless, in the absence of a direct benefit, the study may advance the fields by provided an increased understanding of the utility of CYP2C19 genotyping for PPI dosing. Such understanding is deemed essential for broader dissemination of genotyping into clinical care.

10. Conflict of Interest:

Publications that may result from such research can enhance the reputation of the investigators.

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Nemours Protocol and Statistical Analysis Plan

08/03/2016

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The Implementing Genomics in Practice (IGNITE) consortium (RFA-HG-12-006, RFA-HG-12-007 and RFA-HG-13-004) was created to enhance the use of genomic medicine. Collaborative efforts between three groups, UF and Vanderbilt University (two groups funded within the IGNITE network) and Nemours Children's Health System (an IGNITE affiliate member institution), highlight the impact of the IGNITE network in pulling together these groups and creates an outstanding paradigm for further advancing the network by extending to the IGNITE affiliate members.

ABSTRACT

Proton Pump Inhibitors (PPIs) are among the most commonly prescribed drugs in the world and are the agents of choice for the treatment of GERD and related conditions. While effective, PPI treatment fails in 40% of adults and children. The efficacy of PPIs is highly dependent on plasma concentrations achieved following drug administration. All PPIs are metabolized by the CYP2C19 enzyme located in the liver, which is encoded by the highly polymorphic CYP2C19 gene.

Depending on the CYP2C19 genotype individuals are classified into different phenotypes: poor metabolizer (PM), intermediate metabolizers (IM), normal metabolizers (NM), rapid metabolizer (RM) and ultra-rapid metabolizers (UM). Genetic variants in CYP2C19 are known to profoundly influence PPI plasma concentrations and therefore, response to PPI therapy. In addition to inadequate efficacy in some patients, short- and long-term use of PPIs has been associated with a number of significant adverse effects, including increased risk of intestinal and pulmonary infections, bone fractures and acute and chronic kidney disease, which may be influenced by CYP2C19 genotype.

Some of these adverse effects have been linked to increasing gastric acid suppression or higher than conventional PPI doses, and one randomized trial of PPI treatment for asthma reported a higher risk of respiratory infections among children phenotyped as IMs and PMs compared to NMs, RMs and UMs. These data support the hypothesis that genotype-guided dosing of PPIs would reduce treatment failures in RMs and UMs and minimize PPI-associated infections (and perhaps other adverse effects) in IMs and PMs. The overarching goals of this project are to use longitudinal, electronic health record (EHR) data to add to the body of evidence on PPI efficacy and safety by CYP2C19 genotype, and to document the feasibility and potential clinical benefits of CYP2C19 genotype-supported PPI dosing.

The overarching hypotheses are that PPI-associated adverse events are related to CYP2C19 variation and that genotype-supported dosing will reduce the risk of adverse events compared to conventional PPI dosing in adults and children. The aims of this project are (1) to define clinical outcomes in PPI-treated patients based on CYP2C19 genotype in a cross-network collaboration with Vanderbilt in 10,500 PPI-exposed patients with known CYP2C19 phenotypes; (2) to implement pharmacogenetic testing of the

CYP2C19 gene-PPI drug pair in adults at UF Health and in children at Nemours Children's Health System in the context of a comparative effectiveness genotype-supported vs. conventional PPI dosing trial; (3) In collaboration with Vanderbilt, using genotype and phenotype data in the EHR to conduct a phenome-wide association study (PheWAS) on CYP2C19 genotype-inferred phenotype.

SPECIFIC AIMS

Proton Pump Inhibitors (PPIs) are indicated for the treatment of gastroesophageal reflux disease (GERD), H.pylori infection, peptic ulcer disease, and gastric protection in patients at risk for gastrointestinal bleeding. PPIs are among the most commonly prescribed drugs in the world and their most common indication is GERD.

Refractory GERD is the term used to describe non-responsiveness to conventional PPI therapy, and it occurs in up to 40% of patients (both children and adults) even when the PPI dose is doubled. The efficacy of PPIs is highly dependent on plasma concentrations achieved following drug administration. All PPIs are metabolized in part by the CYP2C19 enzyme located in the liver, which is encoded by the highly polymorphic CYP2C19 gene.

Depending on the CYP2C19 genotype, individuals are classified into different metabolizer phenotypes: poor metabolizers (PM, 2 loss-of-function CYP2C19 alleles); intermediate metabolizers (IM, one loss-of-function allele); normal metabolizers (NM, no loss or gain-of-function alleles); rapid metabolizer (RM; one gain-offunction allele) and ultra-rapid metabolizers (UM, two gain-of function-alleles). Genetic variants in CYP2C19 are known to profoundly influence PPI plasma concentrations and therefore, response to PPI therapy. For example, individuals classified as either RM or UM have lower PPI concentrations compared to NM or of loss-of- function (LOF) allele carriers, respond poorly to PPI therapy and some fail to respond even when the PPI dose is doubled.

Short- and long-term use of PPIs has been associated with a number of significant adverse effects, which may be influenced by CYP2C19 genotype. Examples of serious adverse effects associated with PPIs included: 1) vitamin/mineral malabsorption associated with increased risk of bone fractures (calcium deficiency); 2) increased risk of intestinal infections (Clostridium difficile and small bowel bacterial overgrowth) and pulmonary infections (community and hospital-acquired), and 3) acute interstitial nephritis, chronic kidney disease and end stage renal disease; Some of these adverse effects have been linked to increasing gastric acid suppression or higher than conventional PPI doses. In a recent study reported by Dr.Lima, children taking lansoprazole to treat poorly controlled asthma had a higher risk of infection compared to placebo, and the increased risk of infection was observed in IMs and PMs but not in NM, RM or UM patients.

These data support the hypothesis that genotype-guided dosing of PPIs would reduce treatment failures in RMs and UMs and minimize PPI-associated infections (and perhaps other adverse effects) in IMs and PMs. The overarching goals of this project are to use longitudinal, electronic health record (EHR) data to add to the body of evidence on PPI efficacy and safety by CYP2C19 genotype, and to document the feasibility and potential clinical benefits of CYP2C19 genotype-supported PPI dosing.

Aim1: Define clinical outcomes in PPI-treated patients based on CYP2C19 genotype. This aim will be conducted as a cross-network project with Vanderbilt, the other IGNITE site with substantial CYP2C19 genotype data in the EHR. Between the two institutions, we have CYP2C19 genotype data in the EHR on more than 16,000 patients, of whom more than 10,000 have exposure to a PPI. This provides significant power to test the influence of genotype on clinical outcomes with PPI use. The hypothesis is that PMs and IMs will have better control of GERD, but increased rates of adverse events (infection, bone fractures, adverse effects on kidney). In contrast, rapid metabolizers (RM and UMs) are more likely to develop refractory GERD and lower rates of PPI-related complications.

Aim 2: Implement pharmacogenetic testing of the CYP2C19 gene-PPI drug pair in adults at UF Health and in children at Nemours Children's Health System in the context of a comparative effectiveness genotype-supported vs. conventional PPI dosing trial. Given that PPI efficacy and adverse events are related to PPI exposure and to metabolizer phenotype, individualizing treatment using CYP2C19 genotype guided dosing is expected to improve patient outcomes. We hypothesize that genotype-guided PPI dosing will reduce the rates of infection, fracture, adverse kidney outcomes and evoke equivalent efficacy for GERD symptoms compared to conventional dosing in children and adults referred to Nemours Children's Hospital and UF Health. We will evaluate physician acceptance of genetic information into clinical practice and potential impact on clinical outcomes.

Aim3. Using genotype and phenotype data in the EHR, conduct a phenome-wide association study (PheWAS) on CYP2C19 genotype-inferred phenotype. We will conduct this aim in collaboration with Vanderbilt University, who will lead the aim. Please see their proposal for specific details.

SIGNIFICANCE

Currently, use of CYP2C19 genotype to guide PPI dosing is not recommended by either the American Gastroenterology Association or the American College of Gastroenterology. At UF Health, gastroenterologists have not used CYP2C19-guided PPI dosing and thus there is a need for education, further evidence generation, and a pilot implementation study. Nemours Children's Hospital has implemented a program whereby pediatric gastroenterologists can utilize CYP2C19 genotype-guided PPI dosing in children with GERD. The proposed study is significant because it may extend the evidence regarding influence of CYP2C19-genotype in PPI efficacy and safety, lead to more rationale dosing of PPIs, increase recognition of potential adverse events of PPI therapy and the ability to minimize them with genotype guided dosing, and build a body of evidence to facilitate the implementation of pharmacogenetic-guided PPI dosing.

GERD Refractory to PPI Treatment. Gastroesophageal reflux disease (GERD) affects 10-20% of adults and children in Western countries [1,2]. PPIs are agents of choice to treat GERD, and are among the highest-selling classes of drugs in the US with $9.5 billion in sales in 2015. Six PPIs are commercially available: omeprazole (Prilosec); lansoprazole (Prevacid), dexlansoprazole (Dexilant) esomeprazole (Nexium), pantoprazole (Protonix), raberprazole (Aciphex). Low doses of omeprazole and lansoprazole can be purchased over the counter. The use of PPIs has increased dramatically during the past decade[3] owing in part to the perception among clinicians that PPI treatment is safe and causes negligible side effects[4-7].

However, there are increasing safety concerns expressed by the FDA and others regarding the use and safety of PPIs in children[8] and adults.[9,10] While effective for symptom relief and erosive esophagitis, PPI treatment fails in up to 40% of adults and children with GERD[11]. Failure to respond to standard, once daily PPI treatment or complete or partial response to twice daily PPI treatment has been defined as refractory GERD[1,12]. Several factors have been associated with refractory GERD including adherence to PPI therapy, esophageal hypersensitivity and inter-individual differences in PPI metabolism[11]. The present proposal focuses on differences in PPI metabolism as a mechanism underlying response to GERD symptoms and refractory GERD.

CYP2C19 genetic variation influences the pharmacokinetics of PPIs. PPIs inhibit the final pathway of acid production in gastric parietal cells by covalently binding to the H+/K+-ATPase proton pump leading to inhibition of acid secretion, which lasts for 24-48 hours despite the drug's short pharmacokinetic half-life[13-15]. Most PPIs are metabolized by CYP2C19 and CYP3A4; have short elimination half-lives (1-4 hours) and distribution volumes that are 8%-20% of total body water. All PPIs have low hepatic clearances, low first-past metabolism and have high bioavailabilities[6]. The CYP2C19 gene that encodes the CYP2C19 enzyme is located on chromosome 10q24.1-q24.3, has 9 exons and is highly polymorphic[16-18]. Several loss of function alleles (e.g., CYP2C19*2 through *9) reduce drug clearance and significantly increase PPI plasma concentrations and area under the plasma concentration-time curve (AUC). Carriers of one or two loss of function (LOF) alleles are referred to as intermediate and poor metabolizers (IMs and PMs), respectively.

The extent to which CYP2C19 variation affects clearance depends on the fraction of total clearance attributable to CYP2C19. All PPIs undergo significant metabolism by CYP2C19 and genetic variation in CYP2C19 influences the pharmacokinetics of all PPIs.[6,19,20]. The G681A polymorphism causes a splicing defect in exon 5 resulting in protein synthesis termination and is responsible for the *2 allele, the most prevalent LOF allele. The wild type (WT) CYP2C19*1 allele is the nomenclature assigned when no functional variant alleles are present, and is associated with a more rapid clearance and lower AUCs compared to the PM or IM phenotypes.

Those who are homozygous for *1 are referred to as and are responsible for normal metabolizer (NM) phenotype[17]. CYP2C19*17 is a gain- of-function allele that increases the clearance of PPIs and reduces AUC compared to NMs. The *1/*17 or *17/*17 genotypes contribute to the rapid or ultra-rapid metabolizer phenotype (RM or UM, respectively) [21,22]. The AUC following the administration of equal PPI doses can vary 3-10 fold between PMs and UMs.[6,23-26] ***Table 1*** shows population frequency data for the various genotypes/inferred phenotypes from the UF clinical implementation of CYP2C19 genotyping for clopidogrel response prediction.

TABLE 1. CYP2C19 genotypes, inferred phenotypes, and population frequencies

ALLELE[a]	GENOTYPE/ACTIVITY	PHENOTYPE[b]	FREQUENCY
*1/*1	Wild Type (WT)/ 2 active alleles	NM	39%
*1/*n	Heterozygous WT/1 active, 1 LOF allele	IM	20%
*2/*2 or *n/*n	Homozygous variant/2 LOF alleles	PM	1.7%
*1/*17	Heterozygous WT/1 active, 1 GOF allele	RM	28%
*17/*17	Homozygous variant/2 GOF alleles	UM	5.3%
*2/*17	Heterozygous; 1 LOF/ 1 GOF allele	?	5.6%

[a] *2, *3, *8, or *9 refer to loss-of-function (LOF) alleles, n refers to any of the LOF alleles

[b] Abbreviations: GOF –gain of function; NM—normal metabolizer; IM – intermediate metabolizer; PM – poor metabolizer; RM—rapid metabolizer; UM—ultra-rapid metabolizer

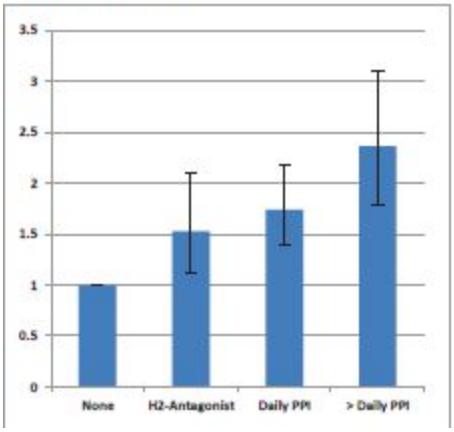
Given that the frequencies of homozygous variants are relatively rare, our analyses will focus on three main phenotype groups from the five CYP2C19 phenotypes listed in ***Table 1***. These will be those individuals carrying a LOF allele (hereafter referred to as PMs), those with no variant alleles (hereafter referred to as NMs) and those carrying a gain of function allele (hereafter referred to as RMs). As noted, those who are *2/*17 will not be included in the primary analysis since the relevant phenotype is not clear. In secondary analyses we will associate outcomes with the five phenotypes listed in table 1.

Efficacy of PPIs depends on plasma concentrations and CYP2C19 genetic variation. Studies in adults and children report that the efficacy of PPIs to treat GERD and related conditions are closely linked to plasma concentrations [27-30]. Numerous studies in adults have shown that CYP2C19 variants markedly influence the pharmacokinetics and pharmacodynamics of PPIs (see reviews 6,16-18,23,31-33). H pylori cure rates are significantly higher among PMs compared to RMs [24,25] and higher doses of PPI are required to treat H pylori in RMs[25,34]. There are fewer studies in children. Although earlier studies suggested that CYP2C19 variants had little effects on PPI PK in children, recent studies support a role for CYP2C19 variants[22,35,36].

Long-term PPI use is associated with increased risk of adverse events. PPIs are among the most commonly prescribed drugs in the world owing to their efficacy and perceived safety. However, there is mounting evidence that long-term use of PPIs are associated with a number of significant adverse effects including gastric and respiratory infections, bone fractures and kidney disease[10,37]. Additionally, there is evidence suggesting that higher daily PPI doses and/or the degree of acid suppression increase risk, thereby supporting a role for CYP2C19 genetic variation contributing to adverse events.

Infections Chronic gastric acid suppression reduces the effectiveness of the pH barrier in the stomach to protect against infections from ingested bacteria and viruses, and alters the gastric flora of the gastrointestinal tract. [38,39] Studies in adults and children have reported increased risk of intestinal infections with Salmonella, Clostridium difficile, Shigella, Campylobacter, Streptococcus, Lactobacilli and fungus (see reviews40-46). Importantly, the dysbiosis associated with PPI depends on the dose or degree of acid suppression. In a pharmacoepidemiologic study comparing the risks of nosocomial Clostridium difficile infection (CDI) among patients receiving gastric acid suppression treatment, increasing the intensity of suppression (no suppressive treatment; H2 antagonists (HTA), daily PPI and twice or more PPI administration/day) was associated with increased CDI 47 (See ***Figure 1***).

Figure 1. Odds ratio for CDI with gastric suppression therapy



If aspirated during reflux episodes, infected gastric media may increase the risk of upper respiratory infections [48,49]. A prospective study of 46 children with chronic cough or wheezing demonstrated the association of nonacid reflux with higher rates of positive lung bronchoalveolar lavage cultures in children [50]. Additionally, acid suppression therapy in children resulted in bacterial overgrowth including Staphylococcus and Streptococcus, and full-column non-acid reflux was associated with bacterial overgrowth in the lung, which was proportional to acid suppressor dose[50,51]. Several studies have reported associations between the chronic use of PPIs and upper respiratory infections and community-acquired pneumonia in infants, children and adults [40,52-60].

Additionally, the risk of community-acquired pneumonia was associated with higher PPI doses [58]. A recent, randomized, placebo controlled clinical trial of the efficacy of lansoprazole (LZ) to improve asthma symptoms in 300 children with poorly controlled asthma reported increased prevalence of upper respiratory infection, sore throat and bronchitis in participants taking LZ compared to placebo [61]. In an ancillary study we documented that children with asthma receiving LZ and phenotyped as PM had a 3-fold higher incidence of respiratory infections compared children who did not carry a LOF allele[62]. Specifically, the risks of upper respiratory infections, OR=2.96 (95% CI, 0.86-2.79; $p < 0.05$) and sore throat, OR=2.94 (95% CI, 1.23-7.05; $p < 0.05$), were higher among PMs compared to other phenotypes (NM+RM) and to placebo; blood levels of lansoprazole were also higher among PM compared to NM/RM[63]. These data are the first to show that CYP2C19 genetic variation is linked to PPI-associated respiratory infections.

Bone fracture. Vitamin/mineral malabsorption has been associated with increased risk of bone fractures in the elderly [64-66], which in some studies were related to higher daily doses of PPI (See review67). PPI use has been associated with bone fractures in young adults but not in children younger than 18 years old68. However,, Dr. Lima and colleagues, studying the efficacy of lansoprazole in childhood asthma, reported that 5 children in the PPI group reported fractures compared to one child in the placebo arm suggesting that PPI-associated fractures may occur in children[61]. The mechanism underlying PPI-associated bone fractures is not known. However, it may involve acid suppression and increasing gastric pH as H2 antagonist use has also been associated with bone fractures[69]

Acute and chronic kidney disease. Early case and anecdotal reports suggest associations between PPI use and acute kidney injury (AKI)[70-72]. More recent studies link PPI use with the risk of chronic kidney disease (CKD)73,74 and with progression to end-stage renal failure (ESRD) [75,76] Twice -daily PPI dosing was associated with a higher risk of CKD compared to once-daily dosing[74] supporting the idea this adverse event is dose related. Interestingly, a population-based cohort study reported hazard ratios for the risk of kidney injury were 2.9, 2.6, 2.5 and 2.4 for omeprazole, lansoprazole, rabeprazole and pantoprazole, respectively[73], thus demonstrating similar propensities among PPI to associate with AKI[73]. The same study estimated the risk of drug rechallenge among patients with AKI following initiation of PPI treatment. PPI-associated kidney disease presents a real dilemma for physicians especially in treating elderly patients who require acid suppression treatment. Our study is important because it proposes that PP-associated kidney disease may be minimized by genotype-guided dosing.

The primary adverse effect phenotypes for focus in this proposal will be increased rate of gastrointestinal and pulmonary/upper respiratory infections, bone fracture and adverse kidney outcomes. These have been selected for focus because there is strong association data between PPI use (and PPI dose) and these adverse phenotypes, they have been observed in the age ranges that will represent the majority of our population, and they are computable phenotypes.

While other adverse effects such as suggestions of risk of myocardial infarction, and dementia have been associated with PPIs, we will not focus on these outcomes as the power in our study will not be sufficient to detect these outcomes. Additionally, for dementia the risk has been observed in PPI users over the age of 75, and we are likely to have limited numbers of patients in our analysis of this age group[77,78].

Case for CYP2C19 genotype-guided PPI dosing. The data above provide substantial evidence for the link between CYP2C19 genotype, PPI pharmacokinetics, efficacy and adverse effects. Use of PPIs has increased dramatically in the last decade, driven in part by an impression that they are “benign drugs”. As a result they are often prescribed as long term therapy in multiple different patient populations. It is not uncommon for patients to be on PPI therapy for multiple years or even “indefinitely”, despite no clear indication being present.

As described in this application, long-term PPI use has been associated with potentially serious adverse events. Recent high profile publications have increased awareness among clinicians of potential risks of PPIs, and the need for more rational use and dosing. In many cases data suggest the risk of adverse effects are dose related, which implies that safety of these drugs can be improved through rational dosing based on CYP2C19 genotype. Although the case for pharmacogenetic testing of CYP2C19 to guide PPI therapy has been made recently[62], additional evidence is needed. This project is significant because it will add to the evidence of associations between CYP2C19 genotype and adverse outcomes to PPI therapy and will also prospectively test the hypothesis that genotype-guided PPI dosing is safer than conventional dosing in children and adults. It will also provide practical experience on the clinical implementation ofCYP2C19 genotype-guided PPI dosing.

Advancing the IGNITE network. There are multiple levels at which this proposal advances the IGNITE network, and genomic medicine implementation more broadly. First, this is a collaborative effort between three groups, two groups funded within the IGNITE network (UF and Vanderbilt) and an IGNITE affiliate member institution (Nemours Children's Health System). Such collaborative efforts highlight the impact of the IGNITE network in pulling together these groups and creates an outstanding paradigm for further advancing the network by extending to the IGNITE affiliate members. Such an effort would likely not have occurred in the absence of IGNITE, highlighting the "greater than the sum of its parts" goals for an NIH-funded network. Importantly, the preliminary data for this proposal come from the Nemours investigators, and this mechanism provides a means of exploiting the power created by the IGNITE network to rapidly move forward with enhanced generation of data to support clinical utility and to advance genomic medicine implementation. Additionally, this proposal includes significant representation of pediatric patients, who to our knowledge are not represented in the current IGNITE network portfolio. This proposal also allows for UF and Vanderbilt to expand upon a gene for which they have significant clinical implementation experience, and extend to an additional, very commonly used drug class. Additional evidence that documents potential clinical utility of use of CYP2C19 genotype to guide therapy with multiple drugs will further advance the arguments with payers for the value and logic of a pre-emptive genotyping approach that will have value across multiple different drugs.

(b) Innovation. This proposal is innovative in that we will employ existing clinical CYP2C19 genotype data in the electronic health record, along with the clinical record of over 10,000 patients with genotype and PPI exposure to add significantly to the literature on CYP2C19 genotype and adverse outcome risk. Given conventional study design limitations, major research funders (ie, NIH, PCORI) are increasingly recognizing the promise of novel "big data" approaches to advance biomedical research. Our proposal's technical innovation is in applying a "big data" approach, incorporating EHR, and outcomes data across UFHealth and Vanderbilt to help clarify the relationship with PPI use, CYP2C19 genotype, and clinical outcomes. The conceptual innovation of our proposal lies in the use of "real world" patient data, rather than conventional cohort or RCT data that generally have strict inclusion/exclusion criteria. This allows us to inform treatment in real world patients not represented in RCTs. We will also utilize a pragmatic trial design that will allow us to simultaneously document the feasibility of genotype-guided PPI dosing, and whether such an approach is as effective and safe as usual care, with potential to document the clinical benefits.

(c) Approach

Preliminary Data in support of this proposal

Patients with CYP2C19 genotype and PPI exposure in the EHR at UFHealth and Vanderbilt. At UFHealth and Vanderbilt, more than 16,000 patients to date have undergone CYP2C19 testing, with 10,185 being prescribed a PPI. Of these approximately 20% are PMs (1 or 2 LOF alleles), 41% are NMs, and 33% are RMs (1 or 2 GOF alleles), and approximately 7% with genotype combinations with unknown phenotypes. The goal of this analysis will be to evaluate the relationship with overall metabolism (poor vs normal vs rapid) with clinical outcomes. The hypothesis is that PMs will have increased rates of acid suppression and associated increased rates of bone fractures, infection, and chronic kidney disease and dementia while RMs are more likely to develop refractory GERD (inadequate acid suppression) and lower rates of PPI-related complications.

Lansoprazole pharmacokinetic data and CYP2C19 genotype. Table 2 demonstrates the relationship between plasma drug concentrations and CYP2C19 genotype.

Table 2. Comparison of lansoprazole plasma concentrations by CYP2C19 phenotype.

Phenotype	Alleles	Number Participants	Mean ±LZ Con. ng/ml
NM	*1/*1	11	199±137
IM	*1/*2 or *3	13	303±165
RM	*1/*17	11	168±146
UM	*17/*17	2	156±123
?	*2/*17	4	201±57.1

The data in Table 2 were collected from children with asthma taking a 30-mg lansoprazole (LZ) dose daily for 6 months[61,63]. Blood was drawn 2 hours after the final dose which corresponds to time at which blood levels peak after an oral dose. Note that compared to individuals with the NM phenotype, LZ concentrations were higher in carriers of one loss-of-function allele (*1/*2 or *3; PM) indicating decreased clearance. Carriers of *17 gain-of- function allele (RM) had lower LZ concentrations compared to NMs or PMs, indicating higher clearance. Though the number of participants is small, *17/*17 participants (UM) had the lowest LZ concentrations; and for *2/*17 participants levels were similar to *1/*1 alleles (NM phenotype). These preliminary data support the hypothesis that CYP2C19 variations influence the PK of LZ in children and that CYP2C19 genotype can be used to discriminate metabolizer phenotypes.

Association Between CYP2C19 Phenotype and pH Testing Outcomes in Children taking PPIs. Esophageal pH probe testing is commonly performed in children to assess the efficacy of proton pump inhibitor (PPI) medication therapy for gastroesophageal reflux disease (GERD). We hypothesized that CYP2C19 enzyme GOF phenotype among children who have undergone esophageal pH probe testing while on PPI therapy would be associated with pH probe acid exposure outcomes. Tissue collected during pH probe testing was genotyped in 74 children using Taq Man techniques. Children with the RM phenotype had a poorer response to PPI compared to PM and NM phenotypes (***Table 3***). Our data support that CYP2C19 variants influence response to PPI and suggest that genotype-guided PPI dosing may avoid pH probe testing in some children. We also hypothesize the same would be observed in adults.

Table 3. CYP2C19 phenotype and PPI efficacy				
CHARACTERISTIC	GOF (N=21)*	LOF (N=53)	RR (CI)	P value
Caucasian, %	76	71		NS
Male, %	66	62		NS
Mean Age at pH test, years(SD)	8(4.6)	8(4.9)		NS
Mean PPI Dose(SD), mg/kg	1.26(0.45)	1.00(0.62)		NS
% time pH < 4	5.71	2.67	1.6 (1.1-2.3)	<0.005
Mean Acid clearance (SD)	181(271)	107(158)	2.2 (1.5-3.2)	<0.0001
Mean Number Acid Reflux Episodes(SD) in 24 hrs	25(35)	24(32)	0.71-1.5	NS
*GOF = Gain-of-function--*17 carriers, LOF = Loss-of-function carriers + no variant alleles				

Comparison of CYP2C19 SNP Frequencies in Controls vs. Children Undergoing Fundoplication.

Table 4 shows allele frequencies of CYP2C19 *2 (LOF) and *17 (GOF) in children who were on PPIs and who had undergone fundoplication surgery were compared to controls, who were asthmatic patients who participated in the PPI trial [61]. DNA for genotyping was obtained from saliva samples collected from controls, and for cases was obtained from tissue samples collected from children during undoplication surgery. The minor allele frequency of the *2 (A) allele (PM or IM phenotypes) was underrepresented in the fundoplication cohort compared to controls and the minor allele for the *17 allele (T) (RM phenotype) was over-represented in the fundoplication cohort compared to controls. These data suggest that RMs are more likely to be undertreated with usual doses of PPI therapy and as a result are subjected to invasive management approaches. In contrast, those with the PM phenotype are effectively treated with a usual dose and so are under-represented in the surgical group. These data could be inferred to suggest that genotype-guided PPI dosing could result in dose increases in RMs, which may have the potential to reduce the number of children who would need to undergo fundoplication surgery.

Collectively, these preliminary data provide compelling evidence for the potential clinical utility of CYP2C19-genotype guided dosing by documenting the relationship between CYP2C19 genotype and PPI pharmacokinetics, that response to PPI therapy, based on gastric pH, is influenced by CYP2C19 genotype, and that patients are undergoing risky, invasive procedures who may simply need a higher dose of PPI to overcome their RM phenotype status.

STUDY DESIGN_ *Focus on Aim2 for Nemours Children's Hospital*

Aim 2: Implement pharmacogenetic testing of the CYP2C19 gene-PPI drug pair in adults at UF Health and in children at Nemours Children's Hospital in the context of a comparative effectiveness genotype-supported vs. conventional PPI dosing trial.

Overview. We have designed a comparative effectiveness clinical trial testing conventional PPI dosing with genotype-supported dosing with clinically meaningful outcomes to determine the feasibility of genetic testing in a pediatric and adult setting. The primary outcome is a composite end point of upper respiratory infections pneumonia, acute gastroenteritis, adverse kidney effects and bone fractures.

Feasibility of the Aim 2 study. Each of the lead investigators for the clinical study (Drs. Lima, Franciosi, Nelson and Johnson) have substantial records in the conduct of clinical trials. Specifically, since 1999, Dr. Lima has led the Nemours Asthma Clinical Research Center (ACRC), one of a Network of 18 - 20 ACRCs funded by the American Lung Association (ALA) to perform clinical trials. The ALA ACRCs have completed several large clinical trials of asthma and have published their results in high impact journals including the NEJM and JAMA. The Nemours ACRC was recently cited by the ALA as the most outstanding ACRC for its patient recruitment; cooperation with the Data Coordinating Center and Network PIs; and for its scientific contributions especially in asthma genetics and pharmacogenetics. Additionally, Dr. Lima was selected to lead a recruitment site by AsthmaNet,a network of asthma clinical research centers funded by NHLBI. Dr. Franciosi has extensive experience in esophageal research involving eosinophilic esophagitis and astroesophageal reflux, clinical trials (Anti-IL-5 therapy, Fluticasone and Budesonide treatment, certolizumab pegol and magnetic positioning colonoscopy). He participated in several large multi-site clinical studies of patient-reported outcomes (PedsQL EoE & GI modules), directs the Registry for Eosinophilic Gastrointestinal Disorders and translational research. Dr. Nelson has a long track record of leading multi-site clinical trials for both federal and industry-sponsored protocols, highlighted by two current trials; a large observational cohort study that has enrolled over 10,000 patients (NCT # 01474811) and a PCORI-funded interventional trial that is now enrolling 3,750 patients. In addition, Dr. Nelson leads the UF CTSI multi-site support team that will help provide resources to ensure a rapid activation (irb, clinicaltrial.gov, etc) and efficient recruitment strategies to conduct a high-quality clinical trial. Dr. Johnson has conducted numerous clinical trials, most notable of which are the two NIH Pharmacogenomics Research Network funded trials, which were multicenter clinical trials she led, which enrolled over 2,500 hypertensive patients, into two complex studies (PEAR and PEAR2) and for which over 1,300 patients met all inclusion criteria and completed all aspects of the study. Collectively these three investigators have over 75 years experience conducting clinical trials, and are well equipped to lead the study described here.

The study is also feasible for enrollment over 9 months, with 3 months follow-up based on volume of the clinics at UF and Nemours. Specifically, the three adult GI providers at UF Health who will be participating in this trial will enroll patients from 10 clinics/wk, which represents approximately 150 patients/wk. Of these, approximately 40% of patients are being seen for peptic acid/GERD indications which should produce 50-60 pts/week that meet inclusion criteria. Assuming only half of eligible patients consent to the study, we anticipate enrolling the 350 adults within a 12 week period. For 2014 and 2015 providers in the Division of Gastroenterology at the Nemours Children's Hospital saw 722 and 877 pediatric patients, respectively, who received a prescription for a PPI. For those years1207 and 1525 respective prescriptions were written for a PPI. Currently the Division sees 20-30 pediatric patients/week that meet the inclusion criteria. Assuming we see 20 patients/week, we expect to enroll at least one-third to one-half of them, which would allow enrollment of 250 patients 6-9 months.

Recent data suggests that in a period of 6-9 months, approximately 60 patients will meet criteria and be enrolled in the study at Nemours Children's Hospital (NCH) alone. In a period of one month (June-July 2016), on average, 71% of PPI prescriptions were performed at GI Clinics from the Nemours Health System (i.e LKM, 1717 ORL, MELB, Lake Nona-NCH) and the remaining were prescribed in other clinics such as

LKM Otolaryngology Clinic and others. At NCH, on average, 38 % of the patients prescribed with a PPI therapy are prescribed at NCH GI Clinic alone. (***Table 4***)

Table 4. PPI prescriptions in a month at Nemours, Orlando

	Unique MRNs	PPI prescribed at GI Clinics	PPI prescribed at other clinics*	PPI prescribed at NCH GI Clinic
Week 1	33	24	9	10
Week 2	34	24	10	15
Week 3	25	16	9	10
Week 4	48	36	12	18

*i.e. LKM Pulmonology, MLB Reumatology, NCH Nephrology, NCH Neurology, NCH orthopedics, NCH Otolaryngology, LKM Otolaryngology, NCH, Allergy/Inmmunology, NCH, Plastic Surgery, 1717 ORL Genetics, NCH Nutrition, 1717 ORL Oncology, 1717 ORL Nephrology, 1717 ORL Otoralyngology, others

In order to improve enrollment efforts, Spartan RX genetic testing equipments will be required at Nemours satellite GI Clinics (i.e. LKM, MELB, 1717 ORL).

Study Design. The study is a randomized, open-label, comparative effectiveness clinical trial in 180 patients (60 children, 120 adults) with GERD or any stomach acid mediated condition that will require a PPI. Patients will volunteer to be randomized to either conventional PPI treatment or genotype-supported PPI treatment. All participants will have symptoms of GERD or related diseases who are candidates for PPI therapy or who are on PPI therapy and classified as refractory GERD. Adult patients will be enrolled at UF Health Gastroenterology Clinics and children will be enrolled at the Nemours Children's Hospital Gastroenterology Clinics. After obtaining informed consent, participants will be genotyped and randomly assigned to either conventional treatment or genotype-supported PPI therapy using a randomization table. At NCH, study participants in the control/conventional arm will be blinded on CYP2C19 metabolizer status. Study staff members will not be blinded to any study procedures or determinations as this will improve and facilitate study feasibility. At the end of study period (i.e. 8-12 weeks) study participants in the control/conventional arm will be revealed of metabolizer phenotype status. We will also collect data from the health records for up to 24 months after enrollment on upper respiratory infection (cold), sore throat, strep throat, bronchitis, pneumonia, ear infection, acute sinusitis (sinus infection), bone fractures, renal function, and gastrointestinal infections for preliminary assessment of the effects of CYP2C19-supported PPI dosing on adverse effects.

Participation stipends. Each participant will receive $25 dollars at the end of study participation. Participants must have completed all study procedures by the end of the study period in order to receive the complete study stipend. If a study participant decides to withdraw from study before the end of study period (before 3 months), the participant will receive a pro-rated stipend. A deposit will be provided in a single CT Payer master card. Each participant will be provided a CT Payer (master card) at the moment of consent. The CT Payer card will be uploaded with the stipend at the end of study participation. Participants whom insurance coverage is limited, a separate deposit will be provided to cover study therapy expenses. We expect to incur in study drug expenses for participants with higher PPI dose prescriptions than conventional dosing (i.e. RMs and UMs).

Dosing. All participants will be genotyped and those randomized to the conventional arm will be dosed according to FDA product labeling. At Nemours, for those randomized to genotype-supported arm, the treating clinician will be provided dosing recommendations with the dosing recommendations based on CYP2C19 genotype-inferred metabolizer phenotype. Specifically, for participants classified as PM and IM we will recommend that their conventional (based on NM phenotype) PPI doses be reduced 50% (halved). We will recommend that conventional PPI doses be increased 50% and 100% for RMs and UMs, respectively. For participants with the *2/*17 diplotypes we recommend they be dosed as NMs. At UF Health, the PMP Committee will convene with the GI clinicians to evaluate and approve and or modify the recommendations for adults. Treatment will be 8-12 weeks. At NCH, clinical support in regards to PPI recommended dosing per CYP2C19 metabolizer phenotype will be provided to GI physicians in EPIC (EMR system). High PPI doses will be aligned with current clinical practices by doubling the original dose..

Table 5. PPI dosing guide per metabolizer phenotype

Metabolizer Phenotype	PPI recommended dosing
NM or Inconclusive (Conventional dosing)	**Esomeprazole (Nexium)** **<10 kg: 5 mg daily; 10-20 kg: 10 mg daily; > 20 kg: 20 mg daily** **Lansoprazole (Prevacid)** **<15kg: 7.5mg daily; 15-30 kg: 15 mg daily; > 30 kg: 30 mg daily** **Omeprazole (Prilosec)** **<10 kg: 5 mg daily; 10-20 kg: 10 mg daily; > 20 kg: 20 mg daily** **Pantoprazole (Protonix)** **<10 kg: 5 mg daily; 10-20 kg: 10 mg daily; > 20 kg: 20 mg daily**
PM or IM (50%↓ of Conventional dosing)	**Esomeprazole (Nexium)** **<10 kg: 2.5 mg daily; 10-20 kg: 5 mg daily; > 20 kg: 10 mg daily** **Lansoprazole (Prevacid)** **<15kg: 3.75mg daily; 15-30 kg: 7.5mg daily; > 30 kg: 15mg daily** **Omeprazole (Prilosec)** **<10 kg: 2.5 mg daily; 10-20 kg: 5 mg daily; > 20 kg: 10 mg daily** **Pantoprazole (Protonix)** **<10 kg: 2.5 mg daily; 10-20 kg: 5 mg daily; > 20 kg: 10 mg daily**
RM (50% ↑ of Conventional dosing)	**Esomeprazole (Nexium)** **<10 kg: 7.5mg daily; 10-20 kg: 15 mg daily; > 20 kg: 30 mg daily** **Lansoprazole (Prevacid)** **<15kg: 11.25mg daily; 15-30 kg: 22.5mg daily; > 30 kg: 45mg daily** **Omeprazole (Prilosec)** **<10 kg: 7.5 mg daily; 10-20 kg: 15 mg daily; > 20 kg: 30 mg daily** **Pantoprazole (Protonix)** **<10 kg: 7.5 mg daily; 10-20 kg: 15 mg daily; > 20 kg: 30 mg daily**
UM (100% ↑from Conventional dosing)	**Esomeprazole (Nexium)** **<10 kg: 10 mg daily; 10-20 kg: 20 mg daily; > 20 kg: 40 mg daily** **Lansoprazole (Prevacid)**

	<15kg: 15mg daily; 15-30 kg: 30 mg daily; > 30 kg: 60mg daily **<u>Omeprazole (Prilosec)</u>** **<10 kg: 10 mg daily; 10-20 kg: 20 mg daily; > 20 kg: 40 mg daily** **<u>Pantoprazole (Protonix)</u>** **<10 kg: 10 mg daily; 10-20 kg: 20 mg daily; > 20 kg: 40 mg daily**

<u>CYP2C19 genotyping.</u> Genotyping at UF will be done by the UF Pathology Laboratory, as has been done for over 1,600 patients in the UF Health CYP2C19-clopidogrel implementation, with laboratory results reported in the same way, and clinical decision support built into the EHR in the same manner as was done for clopidogrel, but specific to the PPIs. Nemours Children's Hospital, will genotype using the Spartan RX system. Spartan RX is a novel genotyping platform recently approved by the FDA as an in-vitro diagnostic that will interrogate *2, *3 and *17 CYP2C19 using saliva samples[80]. Two of these rapid turnaround (approx. 1 hour) platforms reside in Nemours Children's Hospital (NCH) in Orlando and are supervised by Dr. Lili Miles, Chair of Pathology and Director of the CLIA approved Clinical Lab.

CYP2C19 genotyping on the Spartan RX system has been validated to meet all CLIA/CAP requirements at Nemours Children's Hospital and the platforms are currently being used in a pilot program funded by NCH to implement PGX testing at NCH with plans for dissemination to all Nemours sites in the Nemours Children's Health System. The Implementation Program is led by the two Nemours co-PIs of this project, Dr Franciosi and Dr Lima.

Additionally, CYP2C19 genotyping will also be performed at the CLIA certified laboratory in the Alfred I DuPont Hospital in Delaware under the supervision of Susan M Kirwin, B.S.. There are two possible scenarios where study samples will be analyzed in Delaware: (1) study saliva sample has been collected at a Nemours Satellite Clinic (i.e. Melbourne, Lake Mary, 1717 Orlando) including Nemours Children's Specialty Care Clinic at Jacksonville, FL (Downtown and South Clinics)- Oragene OGR-575 saliva collection kits from DNA Genotek. Inc, previously used in studies by Dr Lima and Dr Blake, will be used instead of Spartan Rx equipment; and (2) extra saliva sample collected will be analyzed as a back-up system if Spartan Rx provides Inconclusive genotype results. Remaining saliva sample will continue to be stored for future research when consent has been provided. Expected turnaround time is ± 1 week from collection.

<u>Sample storage for future genotyping/sequencing.</u> We will collect an additional saliva (at NCH) or blood sample (at UF) in order to identify less frequent loss- of-function SNPs or for genotyping or sequencing for future research. At NCH, future samples will be stored at the Nemours Center for Pharmacogenomics and Translational Research in Jacksonville FL under Dr Mougey's direct supervision.

Inclusion Criteria. At NCH, boys and girls 5 to 17 years old diagnosed with GERD or any other stomach acid mediated condition for which a PPI treatment is provided, will be considered for the pediatric arm. Patients are currently under a Proton Pump Inhibitor (PPI) therapy or will start a PPI therapy. Parents/legal guardians and or child must have access to internet and a valid email address in order to complete weekly required study forms. For adults, any patient over the age of 17 who presents to GI clinic with GERD symptoms and is either 1) being initiated on PPI therapy or 2) continue to have symptoms despite PPI therapy will be considered eligible for the study.

Exclusion Criteria. This study will exclude participants who have had extensive esophageal or gastric surgery; and any major chronic illness or conditions that in the opinion of the gastroenterologist that would interfere with participation in the study. At NCH, patients with a history of Phenylketonuria (PKU) and patients with a history of previous adverse effects from PPI treatment or sensitivity to aspartame (NutraSweet, Equal) will be excluded from study. In addition, parents/legal guardians who are non-adherent including inability or unwillingness to provide consent or unwillingness of the child to provide assent(when required), who are unable to take study medications, who are unable to communicate via telephone or other device, who do not have access to a computer with internet access; will be excluded from study participation. No pregnancy test will be obtained from girls in childbearing years as PPI therapy has been demonstrated to be not harmful for pregnancy. [83]

Participant Recruitment Methods. Physicians at UF Health participating clinics will be approached by the study team for involvement in the research protocol. Physicians who agree to participate in this study will be educated on the study protocol and the interpretation of CYP2C19 genotyping. Physicians will participate in surveys that assess their opinions and behaviors as it relates to pharmacogenetics. Patients (or in the case of children, the pediatric patient and their parent/legal guardian) will be approached for participation after a clinical assessment has been performed by a GI provider during a GI clinical appointment. GI providers will notify study coordinator on patient eligibility. Study procedures will be performed after obtaining proper consent and/or child assent. .

In addition, the clinic schedule will be reviewed each week for potential participants. If possible, patients or families will be contacted ahead of time to determine their interest in the study, should they qualify for participation.

Genetic results and clinical decision support. Results will be entered in the participant's electronic health record; genotype results for participants in the control/conventional arm will not be made available to patients/families through the secure patient portal in the EMR to maintain blind status. PPI recommended dosing will be made available to GI providers in the EMR for genotype-guided dosing. PPI templates will include phenotype, genotype, dosing recommendations, and educational information [81]. Participants in the conventional arm will be prescribed the conventional PPI dose.

To minimize risks associated with genetic studies, all results of genetic tests and patient data will be stored in a secure database which is accessible only to study investigators. Genotype and phenotype data will be stripped of all identifiers prior to submission to the NIH dbGAP repository.

Assessment of PPI adverse events and efficacy. Our primary outcome will be a directed interview and questionnaire by study personnel at baseline and every following week up to 12 weeks of participation for efficacy of PPI therapy and for adverse outcomes for the study period: upper respiratory infection (cold), sore throat, strep throat, bronchitis, pneumonia, ear infection, acute sinusitis (sinus infection), and gastroenteritis. We have experience using the questionnaire to evaluate the safety of PPI treatment in children[61]. The prevalence of each respiratory and gastrointestinal symptom will be compared in participants receiving conventional vs. genotype-supported dosing. As secondary outcomes, the efficacy and safety (adverse event score) of PPI therapy will be determined from scores from patient and parent-proxy reported outcomes: the GASP-Q, the validated SN-5 Pediatric Sinonasal Symptom Survey and validated PedsQL™ Gastrointestinal Symptoms Module. Efficacy and adverse events questionnaires will be completed via an electronic questionnaire on a weekly basis and stored in REDCap[82].

At Nemours Children's Hospital continuous monitoring on study metrics will be offered by study staff. Study metrics will include Gasp-Q, PedsQl ™ Gastrointestinal Symptoms Module, SN-5 Pediatric Sinonasal Symptom Survey and Safety Q.

- Gastrointestinal problems under scope includes; stomach pain and hurt; stomach upset; food and drinks limits; trouble swallowing; heartburn and reflux; gas and bloating; constipation; diarrhea; worry; medicines and communication will be measured.
- On the other hand; sinus infection; nasal obstruction; allergy symptoms; emotional distress; activity limitations and overall quality of life are some of the metrics to be evaluated for sinus and/or nasal problems.
- Each metric has its own scoring scale. Gastrointestinal problems will range from 0-100 for "Never" to "Almost always", respectively. Sinus and/or nasal problems have a 1-7 scale for "None of the time" to "All of the time", respectively.
- Safety Assessment (adverse event score) of PPI therapy will be scores from a questionnaire comprised of 7 respiratory symptoms: upper respiratory infection (cold); sore throat; strep throat; bronchitis; pneumonia; ear infection; acute sinusitis (i.e. sinus infection)

Monitoring for adverse events will be performed by the treating medical staff, the Principal Investigators, the site investigators, and the research coordinator(s). We will follow the guidance from the Office for Human Research Protections (OHRP) DHHS: Guidance on Reviewing and Reporting unanticipated Problems Involving Risks to Subjects or Others and Adverse Events http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm). Any event that: (1) involves harm, or an increased potential for harm, to one or more participant(s), or others, AND (2) is unexpected, AND (3) is related, or probably related, to study procedures will be reported to the Nemours and or UF IRB within 5 days of discovery, or within 24 hours if an unforeseen death. We will follow FDA guidelines for report of adverse events to the FDA within the required timeframe for the event. Additional standard institutional IRB reporting guidelines for adverse event reporting will also be followed.

There is no Data and Safety Monitoring Board (DSMB) for this project as the medications being prescribed are part of routine clinical care. Additionally, the study is a pragmatic trial design and there are no required actions by the clinicians based on the genotypes returned. All study staff will report any unanticipated problems to the principal investigators who will determine if reporting to the IRB is required. No Investigational New Drug (IND) form will be filled as there is no increased risk of adverse effects by increasing PPI dose based on genotype and our study does meet the exemption criteria for an IND submission as described below: [84]

(i) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

(ii) (ii) If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;

(iii) (iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly 1 D46.0000 increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

(iv) (iv) The investigation is conducted in compliance with the requirements for review by an IRB (21CFR56) and the requirements for informed consent (21CFR 50); and

(v) (v) The investigation is conducted in compliance with the requirements of 21CFR312.7 (Promotion and sale of investigational drugs).

Metrics for assessing success of pharmacogenetic testing in clinical practice. We will test a variety of variables that will provide insight into attitudes and acceptability of pharmacogenetic testing. These will include: 1) surveys of patients and clinicians on attitudes toward pharmacogenetic testing, 2) the percent of the time in the genotype-supported arm when a dosing revision is recommended based on the patient's genotype and the clinician follows it, and 3) percentage of participants agreeing to future use of DNA for research and data sharing.

Statistical analysis. We will have two primary hypotheses tested in this pragmatic clinical trial: 1. Hypothesis: RMs (1 or 2 GOF alleles) in the genotype-supported arm have better GERD control than RMs in the usual care group 2. Hypothesis: PMs (1 or 2 LOF alleles) in the genotype-supported group have fewer adverse effects (composite of URI/GI infections, kidney adverse effects, bone fracture) than PMs in the usual care group. Continuous variables will be compared between the genotype-supported group and the usual care group using t-test, and categorical variables will be compared using the chi-squared test. The hypotheses will be tested by logistic regression analysis to estimate the odds ratios and 95% confidence intervals for GERD control rate in the RM patients between the two arms and for the adverse events between the PM patients between the two arms. Time to-event analysis will be performed using Kaplan Meier method and a Cox regression model. Significant covariates associated with the events will be adjusted for in the multivariable models. All analysis will be performed in SAS 9.4 (Cary, NC).

We will also have a composite analysis that is conducted in the same manner that combines efficacy and safety and tests the hypothesis that genotype supported PPI dosing leads to better PPI efficacy and safety, as determined by composite of refractory GERD, and adverse effects.

Finally, we will evaluate the influence of genotype-supported dosing recommendations and will evaluate the percentage of the time when a dosing recommendation was made and accepted by the treating clinician, to provide practical data on the willingness of clinicians to adopt this approach. We will also collect patient- and provider-level survey data on attitudes about pharmacogenetics testing; using survey tools adapted from those utilized by other IGNITE groups. These will be shared for network-wide analyses, but because of the number of patients we will study, we should also have sufficient data to perform analyses on our patients along.

Power calculation. Briefly, with 600 participants in the trial, we will have 80% power at alpha level of 0.05 to detect a relative risk of 1.35 or higher in the GERD control within RM patients in the usual care vs. the genotype-supported arm. This assumes that among the 600 participants, 33% will have the RM phenotype, and half will be randomized to each arm of the study. For the adverse event hypothesis, with 600 patients, we expect to have 178 PM patients, with 89 patients in each arm. At alpha level of 0.05, we will have 80% power to detect relative risk reduction of 29% or more in the adverse events for PM patients in the genotype supported group compared to those in the usual care group.

Clinical Trials.gov. This trial requires registration in ClinicalTrials.gov.

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