STUDY OF PHARMACOGENOMIC-GUIDED TACROLIMUS DOSING AND MONITORING IN KIDNEY TRANSPLANT RECIPIENTS

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Funding Sponsor:	UNC Eshelman School of Pharmacy Center for Pharmacogenomics and Individualized Therapy Genetic Medicine Building 120 Mason Farm Road Chapel Hill, NC 27599
Study Product:	Tacrolimus – Generic
IRB Study Number:	16-2073
Study Phase:	Postmarketing; Phase 4

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Protocol Revision History

The following describes the revision history for this protocol.

Protocol Version	History of Changes
Protocol Version 1	Initial version approved by the IRB
Protocol Version 2	The following amendments to this protocol were made: • **Tacrolimus dosing:** To meet the current standard of care at UNC, the initial dosing of tacrolimus was changed from 0.3 to 0.2 mg/kg/day for *CYP3A5* expresser and from 0.15 mg/kg/day to 0.1 mg/kg/day for *CYP3A5* nonexpresser; tacrolimus is to be given in two divided doses with a maximum total daily dose of 20 mg/day. Safety data regarding the initial tacrolimus dosing of 0.1 mg/kg/day and 0.2 mg/kg/day are available in the drug's package insert. • **Safety monitoring**: The statement "AEs will be collected following kidney transplantation" was added in Section 9.2 to clarify that AEs will be collected after subjects have received kidney transplantation and are eligible to receive tacrolimus.
Protocol Version 3	A sentence in the table of schedule of activities was added to clarify that assessment of drug-related AEs may be done in person or by phone.

List of Abbreviations

The following abbreviations and specialized terms are used in this study protocol.

Abbreviation or specialized term	Explanation
AE	Adverse event
ADR	Adverse drug reaction
C_0	Trough concentration
CFR	Code of Federal Regulations
CGI	Cancer Genetics Incorporated
CI	Confidence Intervals
CL	Central Laboratory
CLIA	Clinical Laboratory Improvement Amendment
CrCl	Creatinine Clearance
eGFR	Estimated Glomerular Filtration Rate
CPIT	Center for Pharmacogenomics and Individualized Therapy
CRF	Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent-to-treat
PD	Pharmacodynamics
PHI	Protected health information
PID	Participant identification number
PK	Pharmacokinetic
REDCap	Research Electronic Data Capture
SAE	Serious adverse event
SOP	Standard operating procedure
SNP	Single nucleotide polymorphism
UNC-CH	University of North Carolina in Chapel Hill

1 Study Summary

Title	Study of Pharmacogenomic-guided Tacrolimus Dosing and Monitoring in Kidney Transplant Recipients
Short Title	Pharmacogenomic-guided Dosing of Tacrolimus
Protocol Number	16-2073
Phase	Postmarketing; Phase 4
Methodology	This is a study of pharmacogenomic-guided tacrolimus dosing and drug monitoring in kidney transplant recipients. The pharmacogenomic group will partake in a 12-month study comprising of two periods, Genotype-Guided Initial Dosing Intervention and Follow-up. Briefly, patients currently on the waitlist to receive kidney transplantation at UNC-CH who are being seen by the multi-disciplinary kidney transplantation team will be screened for eligibility. At the pre-intervention assessment (Study Day 0), buccal swab samples for genotyping will be collected on all eligible patients who provided informed consent (performed in real time). All buccal swab samples collected will then be de-identified and sent to Cancer Genetics Incorporated (CGI) for *CYP3A5* genotyping. Results of the genotyping test will be incorporated into electronic medical record (EMR) in addition to the genotype-guided dosing recommendation for each patient. The initial tacrolimus dose will be based on genotype: 0.1 mg/kg/day (non-expressers) or 0.2 mg/kg/day (expressers) given in 2 divided doses; maximum total daily dose of tacrolimus is 20 mg/day. Eligible patients who consented to receive genotype-guided tacrolimus dose will enter the pharmacogenomic group and will receive the initial tacrolimus dosing based on genotype results following kidney transplantation (Study Day 1). Subsequent tacrolimus dosing will then be adjusted according to trough concentrations (C_0) and therapeutic target concentrations of 8-10 ng/mL (0 to 4 months), 6-8 ng/mL (5 to 12 months), and 5-7 ng/mL (>12 months). Dose adjustments and therapeutic drug monitoring will be conducted according to standard UNC-CH procedure. Hence, the genotype-guided dosing recommendation for tacrolimus only refers to the initial tacrolimus dose. All patients in the pharmacogenomic group will be followed from Study Day 2 and up to 12 months from the initiation of the first tacrolimus dose to assess long-term outcome (Follow-up Period). Safety and efficacy measures will be evaluated throughout the study per UNC-CH kidney transplant procedure. Age-, race-, and disease-matched patients who had previously received kidney transplantation with standard tacrolimus dosing from 2010 to present will also be asked to give consent for genotyping (historical controls). These patients will be included in the control group and their safety and efficacy data will be collected retrospectively for up to 12 months from the initiation of first tacrolimus dose.
Study Duration	12-month follow up post-transplant
Study Center(s)	Single-center study
Objectives	Investigate the direct correlation of CYP3A5 genotype with tacrolimus trough levels and clinical outcomes. The primary endpoint of this study is to evaluate the proportion of patients reaching target levels (8-10 ng/ml) on Day 3 and Day 7 after kidney transplantation.

Number of Subjects	260
Inclusion/Exclusion Criteria	Inclusion Criteria: All new kidney transplant recipients aged 18 to 65 years who are admitted at UNC-CH and provided informed consent will be included in this study (unless they meet exclusion criteria). Patients will be excluded from participating in the study to receive genotype-guided tacrolimus dosing if he/she meets any of the exclusion criteria described below. • Recipients who did not consent to participate in the study. • Highly sensitized patients (ie, pretransplant T or B cell flow crossmatch positive) • Recipients of ABO incompatible kidney transplant • Recipients with preformed donor-specific antibodies (DSA) • HLA identical kidney transplant • Recipients of non-kidney transplant • Recipients of repeat transplant if they are on immunosuppression at the time of transplant • Patients using medications that have known pharmacokinetic (PK) drug interaction with tacrolimus • Patients in whom tacrolimus therapy is contraindicated
Study Product, Dose, Route, Regimen	All patients who consented will undergo genotype testing prior to transplant. Therapy for oral tacrolimus will be prescribed by the kidney transplant team as part of a standard immunosuppression agent given to kidney transplant recipients starting at 0.1 mg/kg/day (nonexpressers) or 0.2 mg/kg/day (expressers) given in 2 divided doses. The genotype-guided dosing only refers to the initial tacrolimus dose. Subsequent tacrolimus dosing will then be adjusted according to trough concentrations and desired therapeutic concentrations.
Reference therapy	Pharmacogenomic group is compared to age-, race-, and disease-matched patients who received standard dose of tacrolimus as part of their immunosuppression regimen from 2010 to present (historical controls). As the arrival of the donor kidney may come quickly and unexpectedly, it is possible that enrolled subjects may receive kidney transplantation prior to the incorporation of the *CYP3A5* genotype result into EMR. In this case, patients will receive tacrolimus based on current standard tacrolimus dosing (0.1 mg/kg/day given in two divided doses) and will be included in the control group (prospective controls). Hence, the control group will consist of both historical controls and potentially few prospective controls, if the scenario described above occurred.

Statistical Methodology	All analyses will be performed on the basis of the ITT principle. Continuous variables will be summarized as medians with first to third quartiles. Appropriate conventional statistical analyses will be used to assess the primary and secondary endpoints. Categorical data will be compared using $\chi 2$ or Fisher's exact test, as appropriate; some comparisons will require correction for potential confounders such as race/ethnicity; these will be performed using the Cochrane-Mantel-Haenszel test. Continuous variables will be compared using the Wilcoxon test or the t-test. The incidence of an adverse outcome (ie, graft rejection, graft loss, renal impairment, tacrolimus toxicities, and death) will be estimated using Kaplan–Meier curves. Depending on the distribution of the data, time-to-event (ie, time to reach therapeutic target range) analysis will be performed using an appropriate parametric, nonparametric, or semi-parametric survival analysis method.

1 Ethical Considerations

This is a clinical study protocol for a human research study. This study is to be conducted according to United States (US) and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization [ICH] guidelines), applicable government regulations, and Institutional research policies and procedures grounded on the principles found within the Declaration of Helsinki.

This protocol and any amendments will be submitted to a properly constituted Independent Ethics Committee (IEC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IEC/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. The investigator should provide a list of IEC/IRB members and their affiliate to the sponsor.

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients, safety updates, annual progress reports , and any revisions to these documents will be provided to the IRB/IEC by the investigator.

1.1 Subject Information and Consent

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachments 1, 2, and 3 for copies of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IEC/IRB for the study. The formal consent of a subject, using the IEC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

A written informed consent form, which contained all US federally required elements, all ICH-required elements, and Health Insurance Portability and Accountability Act (HIPAA) Authorization information in language that was understandable to the subject, will be given to each subject. The process of obtaining the informed consent will be in adherence with all federal regulations, ICH requirements, and local laws.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form prior to study participation.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient or the patient's legally acceptable representative, and by the person who conducted the informed consent discussion.

2 Introduction

This is a single-center study of pharmacogenomic-guided tacrolimus dosing and monitoring in kidney transplant recipients.

2.1 Background and Rationale

Renal transplantation from either a live or deceased donor has become the preferred treatment for patients with end-stage renal disease because it prolongs survival and improves quality of life (Port 1994; Schnuelle et al. 1998; Tonelli et al. 2011). Tacrolimus is the standard treatment for immunosuppression with kidney transplant patients as tacrolimus is associated with better graft survival and lower incidence of nephrotoxicity compared to cyclosporine (Bowman and Brennan 2008; Webster et al. 2005a; Webster et al. 2005b).

Tacrolimus is a good candidate for pharmacogenomic-guided dosing and monitoring as it displays variable pharmacokinetics and is also a narrow therapeutic index drug, whereby low drug exposure increases risk to kidney rejection and high doses correlate with nephrotoxicity (Birdwell et al. 2015; Hesselink et al. 2014; Provenzani et al. 2013). Reaching and maintaining therapeutic tacrolimus levels is also often problematic despite adjustment of the initial dose to body weight and frequent monitoring of tacrolimus levels. Furthermore, response to tacrolimus is dose-dependent with large intra- and inter-patient variability that has been partly attributed to genetic differences in the *CYP3A5* gene (Birdwell et al. 2015; Hesselink et al. 2014; Provenzani et al. 2013; Staatz, Goodman, and Tett 2010a, b).

The *CYP3A5* gene encodes for the drug-metabolizing enzyme CYP3A5, which is primarily responsible for the hepatic and renal clearance of tacrolimus (Dai et al. 2006). Specifically, the single nucleotide polymorphism (SNP), rs776746, results in a functional variation that substitutes an adenine (A) for a guanine (G) at position 6986 within intron 3 of the *CYP3A5*. A change from A to G at this position creates a cryptic splice site in intron 3 that results in an alternatively spliced isoform that has an insertion in intron 3. This alternative spliced mRNA alters the reading frame and results in a premature termination codon that leads to a nonfunctional protein (Birdwell et al. 2015; Lamba et al. 2012; Staatz, Goodman, and Tett 2010a, b). The nonfunctional CYP3A5 enzyme corresponds to the *CYP3A5*3* allele, while the functional allele is considered *CYP3A5*1*. Patients who have one (*CYP3A5*1/*3)* or both copies of the functional allele (*CYP3A5*1/*1)* are considered *CYP3A5* expressers. Due to high *CYP3A5* expression, patients with *CYP3A5*1/*1* or *CYP3A5*1/*3* genotype may have lower levels of tacrolimus than patients with *CYP3A5**3/*3 genotype, predisposing them to reduced immunosuppressive effects that may lead to acute kidney rejection. On the other hand, patients who have *CYP3A5**3/*3 genotype do not express *CYP3A5* (*CYP3A5* nonexpressers), which can lead to increased risk for toxicities due to supratherapeutic tacrolimus levels (Birdwell et al. 2015; Lamba et al. 2012; Staatz, Goodman, and Tett 2010a, b; van Gelder, van Schaik, and Hesselink 2014). In line with these findings, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has recently published guidelines for *CYP3A5* genotyping and tacrolimus dosing (Birdwell et al. 2015).

The *CYP3A5* 6986A>G (rs776746) variant is ideal for a pharmacogenomic study as it has a high estimated allele frequency across various populations, including Whites (82% to 95%), Japanese (85%), Southeast Asians excluding Japanese and Chinese (67%), Chinese (65%), Pacific Islanders (65%), Southwest American Indians (40%), and African Americans (33%) (Lamba et al. 2012). The *CYP3A5* 6986A>G genotype has been consistently associated with tacrolimus clearance and trough levels (Macphee et al. 2005; Macphee et al. 2002; Quteineh et al. 2008; Renders et al. 2007; Thervet et al. 2003; Zhang et al. 2005; Zheng et al. 2003). In a recently published review and meta-analysis, the functional CYP3A5*1 allele was associated with higher risk of acute rejection and chronic nephrotoxicity (Rojas et al. 2015).

Whether pharmacogenomic-guided dosing leads to improved clinical outcomes is yet to be determined A prospective study conducted in France showed that genotype-guided dosing resulted in quicker time to reach therapeutic tacrolimus levels but did not demonstrate improved clinical outcomes compared to standard dosing (Thervet et al. 2010). Furthermore, a recent study in Netherlands did not find any difference in the incidence of acute rejection or in the proportion of patients with sub or supratherapeutic tacrolimus levels when comparing subjects who received genotype-guided vs. standard tacrolimus dose (Shuker et al. 2016). Of note, a difference in tacrolimus trough levels and dose requirement was observed between *CYP3A5* expressers and nonexpressers throughout the 3-month period (Shuker et al. 2016). In

both these studies, the population was primarily low-risk (living donor recipients) and was mostly Caucasian (90%).

To further investigate if these results are relevant in the US kidney transplant population, particularly in centers comprising of at least 40% African Americans who are likely to carry the *CYP3A5*1* (expressers) allele and be under-dosed, we are proposing a prospective, single-center study in the US that is aimed at evaluating the relationship between genotype-guided tacrolimus dosing, drug concentrations, and clinical outcomes. Unlike the study population in both European studies, the transplant recipients here at the University of North Carolina in Chapel Hill (UNC-CH) comprise of at least 40% African Americans, with majority of the kidney transplant recipients (75%) receiving their kidneys from deceased donors and are therefore more likely to be at risk for poorer outcomes. Unlike the standard tacrolimus dosing and target tacrolimus levels used by Thervet and colleagues in France, the tacrolimus dosing algorithm and therapeutic target levels being used at UNC-CH is more representative of the regimen for tacrolimus in the US kidney transplant recipients. Additionally, unlike the immunosuppressant regimen used by Thervet *et al* and Shuker *et al*, the immunosuppressant regimen that are currently being used at UNC-CH are representative of the current therapeutic management of US kidney transplant recipients. The standard tacrolimus dosing algorithm currently being used at UNC-CH was developed in accordance to current clinical guidelines and will be considered in the study as standard therapy. To provide a comparable pharmacogenomic-guided algorithm, the genotype-guided dosing algorithm described in Section 6.2.1 is similar to the one that was used in France (Thervet et al. 2010) and Netherlands (Shuker et al. 2016). Hence, the results of this study will provide more relevant findings for the US kidney transplant population as well as provide a reasonable comparison from previous and recently published pharmacogenomic studies in Europe.

In addition, the results of this study will allow us to explore the feasibility of using genotype information for clinical decision making as well as the economic impact of using a genotype-guided treatment algorithm at UNC-CH kidney transplant unit.

2.2 Previous Clinical Experience with Genotype-Guided Tacrolimus Dosing

There are two large prospective pharmacogenomic studies that have tested the efficacy and safety of two genotype-guided tacrolimus doses: 0.15 mg/kg/day for *CYP3A5* expressers and 0.3 mg/kg/day for *CYP3A5* nonexpressers (Shuker et al. 2016). In both studies, there was no difference in safety outcomes between subjects who received standard tacrolimus dosing (0.2 mg/kg/day) versus those who received genotype-guided dosing (0.15 or 0.3 mg/kg/day). There were no significant between-group differences in the overall incidence and severity of biopsy-proven acute rejection (BPAR), rate of acute and delayed graft rejection, and incidence of adverse events, including infection, tacrolimus-associated nephrotoxicity, and post-transplant diabetes mellitus.

Additionally, the genotype-guided dosing recommendation used in previous studies (Shuker et al. 2016), which is similar to the one proposed in this study protocol, follows the recommendation outlined in the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline (https://www.pharmgkb.org/guideline/PA166124619). CPIC's genotype-guided dosing recommendation for tacrolimus was classified as "strong" based on a combination of preclinical functional and clinical data as well as on some existing disease-specific consensus guidelines.

Taken together, in addition to fact that genotype-guided dosing will only be given ONCE at the initial dosing of tacrolimus (subsequent dosing will then be based on tacrolimus trough levels and desired therapeutic concentration in accordance to standard procedure), the investigators do not anticipate any additional risks for subjects who will receive genotype-guided therapy compared to the standard tacrolimus dose that would have been given as part of their immunosuppressant regimen in the event that subjects do not consent to be included in the study.

3 Study Objectives

The primary objective of this study is to investigate the direct correlation of *CYP3A5* genotype with tacrolimus trough levels and clinical outcomes. To meet this objective, a comparison of the primary and

secondary endpoints will be conducted between kidney transplant recipients who underwent prospective pharmacogenomic-guided tacrolimus dosing strategy (pharmacogenomic group) and patients who received standard tacrolimus dosing regimen historically and prospectively (control group). Patient data from the control group will be obtained from 2010 until the end of study period.

The secondary objective of this study is to assess the economic benefit of genotype-guided tacrolimus dosing as measured by direct healthcare cost.

Additionally, we will store banked DNA for future unspecified research from individuals who agree to participate in this study that are undergoing kidney transplantation at UNC-CH. A pharmacogenomic database will be created and maintained in a secure database for possible use in future studies to retrospectively correlate pharmacogenomics (SNPs and patterns of polymorphisms) with therapeutic drug monitoring levels and clinical metrics (eg., Quality/ Safety Metrics – mortality, morbidity, and other quality markers (eg., length of hospital stay, readmission rate etc). We plan to use genomic samples to base clinical care on genetic variants with known functional impact (ie, CYP2D6, TMPT, G6PD etc.). We therefore propose to study other smaller effect loci identified by association studies using the data for hypothesis generating analysis to correlate these single gene polymorphisms and polymorphism patterns with clinical outcomes by creating and then using a pharmacogenomic database.

3.1 Primary Endpoint

The primary endpoint of this study is to evaluate the proportion of patients reaching target levels (8-10 ng/ml) on Day 3 and Day 7 after kidney transplantation.

3.2 Secondary Endpoints

The secondary endpoints are described below.

- The incidence of BPAR within the first 3 months, 3-6 months, 6-9 months, and 6-12 months after transplantation
- Time to achieve tacrolimus therapeutic range at 0 to 4 months (8-10 ng/mL)
- Proportion of patients who had dose adjustments and/or drug alteration or addition due to insufficient immunosuppression
- Incidence of acute and chronic renal impairment. Renal function will be assessed using estimated Glomerular Filtration Rate (eGFR). Creatinine clearance (CrCl) may also be calculated as a reference. Patients will be categorized as having either mild (eGFR of 60 mL/min/1.73m^2 to 89 mL/min/1.73m^2), moderate (eGFR of 30 mL/min/1.73m^2 to 59 mL/min/1.73m^2), or severe renal impairment (eGFR <30 mL/min/1.73m^2).
- Incidence of adverse outcomes (ie, graft rejection, graft loss, renal impairment, adverse drug events, tacrolimus toxicities, and death)

3.3 Exploratory Endpoints

The exploratory endpoints relate to the secondary objective of this study and are described below.

- Direct and indirect cost related to treatment and management kidney transplant recipients

4 Study Design

4.1 General Design

This is a single-center study of pharmacogenomic-guided tacrolimus dosing and drug monitoring in kidney transplant recipients (Figure 1). The pharmacogenomic group will partake in a 12-month study comprising of two periods, Genotype-Guided Initial Dosing Intervention and Follow-up.

Following screening, acquisition of consent, and genotyping, a comprehensive medical chart review will be conducted for the control group. Twelve-month post-transplant data will be collected in the control group with timepoints that match with the pharmacogenomics group.

Figure 1: Study Design

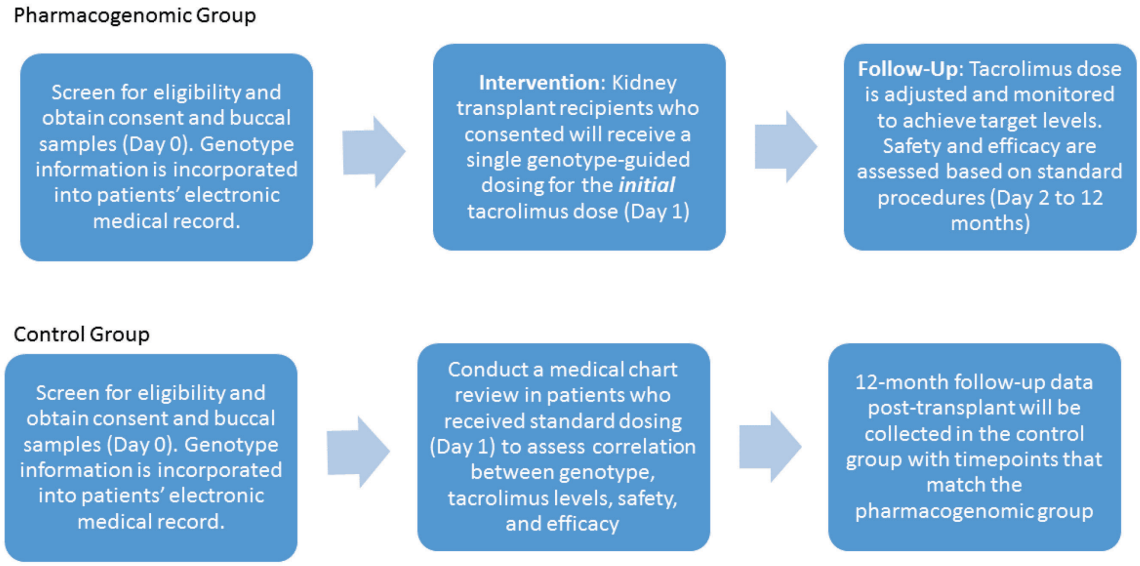

Figure 1. This figure provides the general study design for the study, which comprises of the Screening and two study periods (Intervention and Follow-up) for the pharmacogenomic group. After consent and genotyping have been conducted, a comprehensive medical chart review will be conducted for the control group.

4.2 Rationale

Due to cost, time constraints, and limited patient population (there are approximately 80 kidney transplant recipients at UNC-CH), we are not able to conduct the ideal randomized, double-blind, two-group parallel study. Rather, we are proposing a study design that is practical, given the available resources and still investigates the safety and effectiveness of using genotype-guided tacrolimus dosing.

In this study, a comparison of the primary and secondary endpoints will be conducted between kidney transplant recipients who underwent prospective pharmacogenomic-guided tacrolimus dosing strategy (pharmacogenomic group) and patients who received standard tacrolimus dosing regimen historically and prospectively (control group). Although genotyping the control group can be more expensive and time consuming, knowing the genotype information of the control group will allow us to do the following: 1) identify if the control and pharmacogenomic groups are comparable with regards to genotype; and 2) conduct a subgroup analysis based on treatment and genotype, comparing the outcomes and tacrolimus levels of patients who are *CYP3A5*1/*1, CYP3A5*1/*3*, or *CYP3A5*3/*3* who received standard therapy versus genotype-guided dosing.

4.3 Patient Selection

The study schemas for the pharmacogenomic and control groups are displayed in Figure 2.

Figure 2: Study Schema

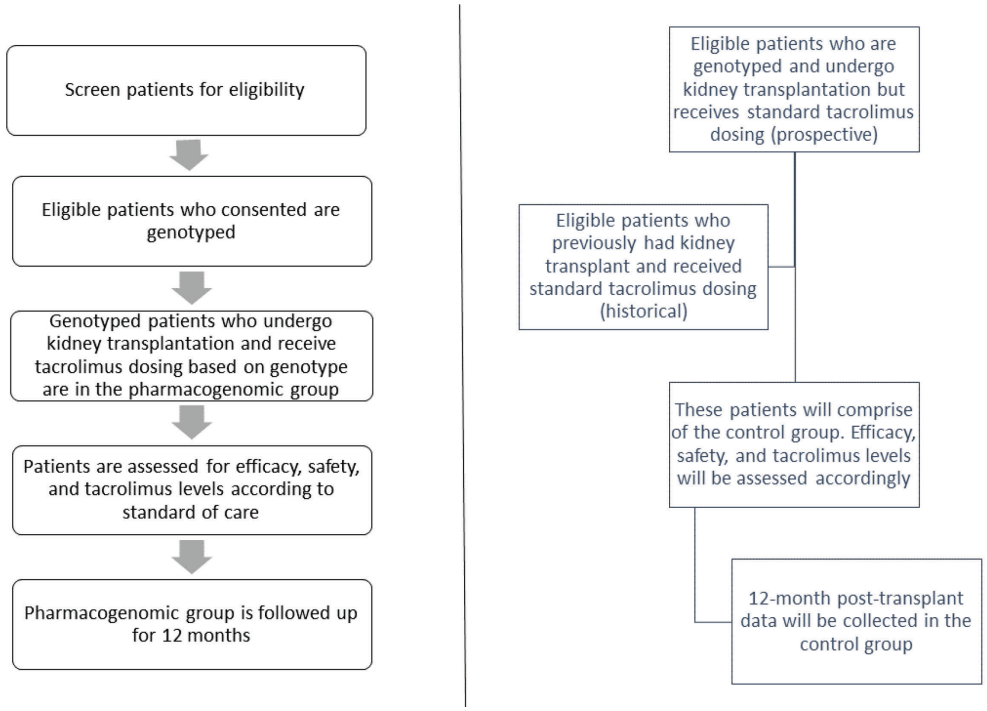

Figure 2. The overall study schematic for the pharmacogenomics group is shown on the left, and the study schematic for the control group is shown on the right.

Briefly, patients currently on the waitlist to receive kidney transplantation at UNC-CH who are being seen by the multi-disciplinary kidney transplantation team will be screened for eligibility. At the pre-intervention assessment (Study Day 0), buccal swab samples for genotyping will be collected on all eligible patients who provided informed consent (performed in real time). All buccal swab samples collected will then be

de-identified and sent to Cancer Genetics Incorporated (CGI) for *CYP3A5* genotyping. Results of the genotyping test will be incorporated into electronic medical record (EMR) in addition to the genotype-guided dosing recommendation for each patient. The initial tacrolimus dose will be based on the pharmacogenomic-guided treatment algorithm described in Section 6.2.1.. Eligible patients who consented to receive genotype-guided tacrolimus dose will enter the pharmacogenomic group and will receive the initial tacrolimus dosing based on genotype results following kidney transplantation (Study Day 1). Subsequent tacrolimus dosing will then be adjusted according to trough concentrations (C_0) and therapeutic target concentrations of 8-10 ng/mL (0 to 4 months), 6-8 ng/mL (5 to 12 months), and 5-7 ng/mL (>12 months). Dose adjustments and therapeutic drug monitoring will be conducted according to standard UNC-CH procedure. Hence, the genotype-guided dosing recommendation for tacrolimus only refers to the initial tacrolimus dose. All patients in the pharmacogenomic group will be followed from Study Day 2 and up to 12 months from the initiation of the first tacrolimus dose to assess long-term outcome (Follow-up Period). Safety and efficacy measures will be evaluated throughout the study per UNC-CH kidney transplant procedure.

As the arrival of the donor kidney may come quickly and unexpectedly, it is possible that enrolled subjects may receive kidney transplantation prior to the incorporation of the *CYP3A5* genotype result into EMR. In this case, patients will receive tacrolimus based on current standard tacrolimus dosing (0.1 mg/kg/day given in two divided doses) and will be included in the control group (prospective controls). Throughout the study, safety and efficacy measures will be evaluated in the prospective controls per UNC-CH kidney transplant procedure. These patients will also be followed from Study Day 2 and up to 12 months from the initiation of the first tacrolimus dose to assess long-term outcome (Follow-up Period).

Additionally, age-, race-, and disease-matched patients who had previously received kidney transplantation with standard tacrolimus dosing will also be asked to give consent for genotyping (historical controls). These patients will also be included in the control group and their safety and efficacy data will be collected retrospectively for up to 12 months from the initiation of first tacrolimus dose. Hence, the control group will consist of both historical controls and potentially few prospective controls, if the scenario described above occurred.

4.4 Genotyping and Dosing Administration

The *CYP3A5* 6986A>G (rs776746) variant is ideal for a pharmacogenomic study as it has a high estimated allele frequency across various populations, including Whites (82% to 95%), Japanese (85%), Southeast Asians excluding Japanese and Chinese (67%), Chinese (65%), Pacific Islanders (65%), Southwest American Indians (40%), and African Americans (33%) (Lamba, 2012). At the UNC-CH multi-disciplinary kidney transplantation team, we see approximately 80 patients for kidney transplantation per year. Of those 80 patients, approximately 60% are Whites and 40% are African Americans. We therefore expect that the *CYP3A5* 6986A>G (rs776746) variant will be relatively frequent in our study population. In addition, to be able to effectively compare outcomes between expressers and nonexpressers as well as between genotyped and nongenotyped subjects, we will make every effort to enroll African Americans to the study so as to get equal numbers of Caucasians and African Americans in our study.

At study enrollment, buccal swab samples will be obtained for genotyping of the *CYP3A5* 6986A>G (rs776746) variant. Based on the genotyping result, patients will be categorized into two categories:

- *CYP3A5* expressers (*CYP3A5*1/*3* and *CYP3A5*1/*1*)
- *CYP3A5* nonexpressers (*CYP3A5*3/*3*)

Patients will then receive the initial tacrolimus dose based on the genotype-guided dosing algorithm outlined in Section 6.2.1. Subsequent tacrolimus dosing will then be adjusted according to trough concentrations and therapeutic target concentrations.

5 Subject Selection and Withdrawal

5.1 Inclusion Criteria

All new kidney transplant recipients aged 18 to 65 years who are admitted at UNC-CH and provided informed consent will be included in this study.

5.2 Exclusion Criteria

Patients will be excluded from participating in the study to receive genotype-guided tacrolimus dosing if he/she meets any of the exclusion criteria described below.

- Recipients who did not consent to participate in the study.
- Highly sensitized patients (ie, pretransplant T or B cell flow crossmatch positive)
- Recipients of ABO incompatible kidney transplant
- Recipients with preformed donor-specific antibodies (DSA)
- HLA identical kidney transplant
- Recipients of non-kidney transplant
- Recipients of repeat transplant if they are on immunosuppression at the time of transplant
- Patients using medications that have known pharmacokinetic (PK) drug interaction with tacrolimus
- Patients in whom tacrolimus therapy is contraindicated

5.3 Patient Recruitment and Screening

Patients receiving pretransplant care for kidney transplantation at UNC-CH will be screened for eligibility based on the inclusion/exclusion criteria described in Sections 5.1 and 5.2. Following acquisition of consent, buccal swab samples will be collected for genotyping. Eligible patients who consented will receive genotype-guided dose for the initial tacrolimus dosing following kidney transplantation. Subsequent tacrolimus dosing will then be adjusted according to trough concentrations and therapeutic target concentrations.

For the historical controls, age- and disease-matched patients who had previously received kidney transplantation with standard tacrolimus dosing will also be asked to give consent for genotyping (historical controls) during one of their post-transplant visit. Eligible patients who consented will be included in the control group and their safety and efficacy data will be collected retrospectively for up to 12 months from the initiation of first tacrolimus dose.

In addition, to be able to effectively compare outcomes between expressers and nonexpressers as well as between pharmacogenomic and control groups, we will make every effort to enroll African Americans to the study, including showing genuine interest to subjects' well-being during one-on-one interactions, referring to them by their last names until given permission to use their first names, and using noninvasive means to collect samples (Johnson et al. 2011; Spence and Oltmanns 2011), so as to get equal numbers of Caucasians and African Americans in our study.

5.4 Removal of Patients from the Genotype-guided Tacrolimus Therapy

Removal of patients from the genotype-guided tacrolimus therapy can occur for the following reasons:

1) Extraordinary medical circumstances: If, at any time the constraints of this protocol are detrimental to the patient's health, then the patient may be removed from the genotype-guided tacrolimus therapy. In this event, the investigator will do the following:
    - Notify the UNC-CH Principal Investigator
    - Document the reason(s) for withdrawal on flow sheets or Case Report Form (CRF)

- Notify the IRB

2) Voluntary withdrawal

3) Excessive toxicity: Patients who experience any grade III or grade IV AE deemed by the Principal Investigator to be related to tacrolimus therapy

4) Investigator's judgment: When deemed in the best interest of the patient by their attending physician, patients should be removed from the study and included in the intent-to-treat (ITT) analysis.

5) Premature closure of the study: This study may be prematurely terminated, if in the opinion of the investigator there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided by the investigator.

6) Circumstances that may warrant termination include, but are not limited to:
   - Determination of unexpected, significant, or unacceptable risk to patients
   - Failure to enter patients at an acceptable rate
   - Insufficient adherence to protocol requirements
   - Insufficient complete and/or evaluable data

6 Study Drug

6.1 Description

Please refer to the current FDA-approved package insert (Attachment 4) for tacrolimus for clinical background information and possible side effects.

6.2 Treatment Regimen

All patients who consented will undergo genotype testing prior to transplant. The genotype-guided dosing recommendation as shown in **Figure 3** below only refers to the initial tacrolimus dose. Subsequent tacrolimus dosing will then be adjusted according to trough concentrations and desired therapeutic concentrations. Therapy for oral tacrolimus will be prescribed by the kidney transplant team as part of a standard immunosuppression agent given to kidney transplant recipients starting at 0.1 mg/kg/day (nonexpressers) or 0.2 mg/kg/day (expressers) given in 2 divided doses; maximum total daily dose of tacrolimus is 20 mg/day.

As the drug and the doses being used are not investigational, tacrolimus will be stored and dispensed in accordance to current UNC-CH pharmacy procedures. The patients and investigators will not be blinded in the study, hence there will be no procedure related to blinding of study drug.

6.2.1 Pharmacogenomic-guided dosing algorithm for the initial tacrolimus dose

The pharmacogenomic-guided algorithm is described in **Figure 3** below. The genotype-guided dosing recommendation only refers to the initial tacrolimus dose. Subsequent tacrolimus dosing will then be adjusted according to trough concentrations and desired therapeutic concentrations.

Figure 3: Pharmacogenomic-guided Dosing Schematic

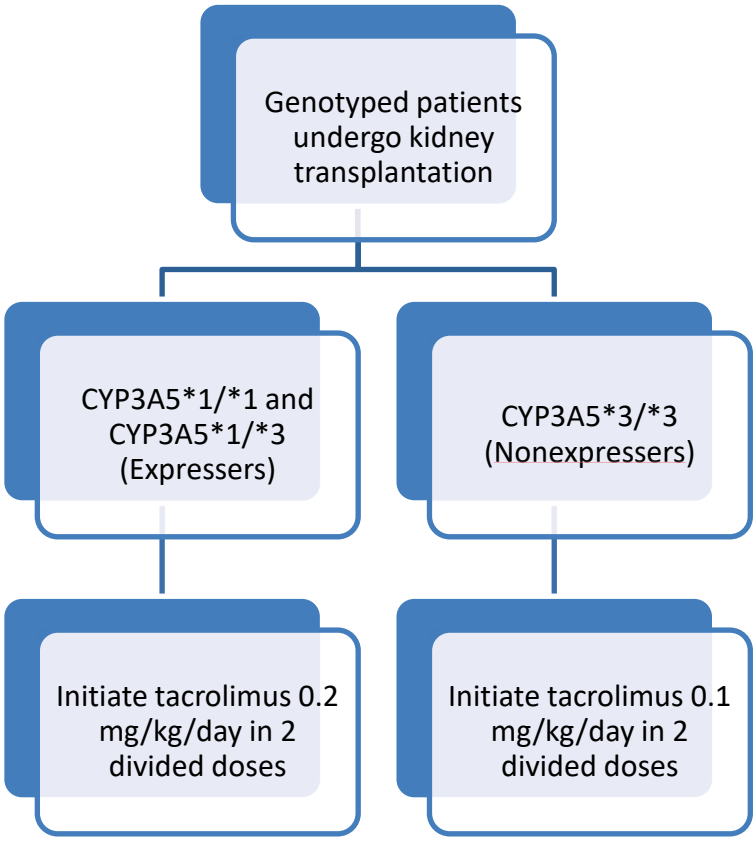

Figure 3 illustrates the genotype-guided dosing for the CYP3A5 expressers and nonexpressers for the initial tacrolimus dose following kidney transplantation.

6.2.2 Therapeutic Target Concentrations

The drug will be adjusted and monitored using current UNC-CH standard kidney transplantation treatment procedure to reach the following therapeutic target levels:

- 8-10 ng/mL during 0 to 4 months of treatment
- 6-8 ng/mL during 5 to 12 months of treatment
- 5-7 ng/mL at >12 months of treatment

Therapeutic drug monitoring, which provides a measure of compliance, will also be conducted according to standard UNC-CH procedure.

7 Study Procedures

Efficacy and safety measures will be assessed throughout the study. All study procedures will be conducted in accordance to applicable regulatory and ethical requirements.

The schedule of events prior to pharmacogenomic intervention and during the Intervention and Follow-up periods are briefly described below and is summarized in **Table 1**.

7.1 Pre-intervention Assessments

The following procedures will be performed at Study Day 0, prior to initiation of the genotype-guided initial tacrolimus dose

- Screening for eligibility
- Acquisition of informed consent
- Buccal swab sample collection
- Hematology, clinical chemistry, urinalysis, eGFR, medical history, and physical exam

- Genotyping

7.2 Pharmacogenomic Intervention

On Study Day 1, Eligible patients who consented will receive genotype-guided dosing only for the initial tacrolimus dosing (0.1 mg/kg/day for CYP3A5*3/*3 or 0.2 mg/kg/day for CYP3A5*1/*1 or CYP3A5*1/*3). In addition, the following safety and efficacy parameters will be collected: hematology, clinical chemistry, urinalysis, adverse event (AE) and renal function assessments, medical history, physical exam, tacrolimus levels, and immunosuppressive and tacrolimus therapy modifications.

7.3 Post-intervention or Follow-up Assessments

Following administration of the initial genotype-guided tacrolimus dose, protocol-driven therapy will cease, and standard of care for tacrolimus dose adjustment and monitoring will be initiated. Efficacy and safety data, including AE assessments, renal function, and tacrolimus levels, will still be collected at each subsequent standard follow-up interval post Study Day 1 and up to 12 months of the initial tacrolimus dose.

Table 1: Schedule of Events

Procedure	Study Day 0	Study Day 1	3-day Follow-up	7-day Follow-up	Week 2 Follow-up	Week 3 Follow-up	Week 4 Follow-up	3-month Follow-up	6-month Follow-up	9-month Follow-up	12-month Follow-up
Screening	x										
Informed consent	x										
Buccal swab sample	x										
Medical history	x										
Drug therapy assessment	x	x	x	x	x	x	x	x	x	x	x
Physical Exam	x	x	x	x	x	x	x	x	x	x	x
Clinical chemistry	x	x	x	x	x	x	x	x	x	x	x
CBCs with differentials	x	x	x	x	x	x	x	x	x	x	x
Urinalysis	x	x	x	x	x	x	x	x	x	x	x
CrCl	x	x	x	x	x	x	x	x	x	x	x
eGFR	x	x	x	x	x	x	x	x	x	x	x
Genotyping	x										
Initiation of genotype-guided dose		x									
Tacrolimus level		x	x	x	x	x	x	x	x	x	x
AE assessments[1]		x	x	x	x	x	x	x	x	x	x
Efficacy assessments[2]		x	x	x	x	x	x	x	x	x	x

Abbreviations: AE=adverse event; BPAR = biopsy proven acute rejection; CBC = complete blood count; CrCl = creatinine clearance; eGFR = estimated Glomerular Filtration Rate.

[1]The AE assessment will be conducted using the AE form (see AE Attachment). AEs may be assessed during an in-person clinic visit or a scheduled phone call.

[2]Episodes of rejections will be assessed as part of the efficacy assessments. Information from biopsies, if collected, will also be assessed.

8 Statistical Plan

8.1 General Considerations

This is a prospective, single-center study aimed to assess the efficacy and tolerability of the initial genotype-guided tacrolimus dosing following kidney transplantation. Proper use of the study design will be ensured by the study investigators informing the study statistician, Dr. Thomas Urban. Additionally, quarterly follow-up on patient accrual will be initiated by the study investigators as further assurance of compliance with the design of the study laid out in this protocol.

The term “pharmacogenomic group” will refer to prospective patients who received genotype-guided dose for the initial tacrolimus dose. The term “control group” will refer to age- and disease-matched eligible patients who received the standard tacrolimus dose after kidney transplantation from 2010 to the end of study period.

8.2 Sample Size Determination

The primary endpoint of this study is to evaluate the proportion of patients reaching target levels (8-10 ng/ml) on day 3 and day 7 after kidney transplantation.

We calculated that a total study population of 260 patients (pharmacogenomics group = 130; control group = 130 patients) would be sufficient to provide a statistical power of 90% to detect a significant difference between the two groups, on the basis of a two-sided $\chi 2$ test, a significance level of 5%, a frequency of approximately 50% for the *CYP3A5*3/*3* genotype, and a 40% and 60% frequency of the primary endpoint in the control and pharmacogenomic group (Thervet et al. 2010), respectively. Because of the nature of the study design we anticipate a small rate of sample dropout (<5%), which should not appreciably impact the power of the study.

8.3 Statistical Methods

All analyses will be performed on the basis of the ITT principle. Unless stated otherwise, the term “descriptive statistics” will refer to the number of subjects, mean, median, standard deviation, minimum, and maximum for continuous data and to frequencies and percentages for categorical data. Categorical variables will be summarized as counts and percentages. All statistical parameters (eg, mean, median, standard deviation) will be tabulated together with their corresponding confidence intervals (CIs) and/or standard errors.

Continuous variables will be summarized as medians with first to third quartiles. Appropriate conventional statistical analyses will be used to assess the primary and secondary endpoints. Categorical data will be compared using $\chi 2$ or Fisher’s exact test, as appropriate; some comparisons will require correction for potential confounders such as race/ethnicity; these will be performed using the Cochrane-Mantel-Haenszel test. Continuous variables will be compared using the Wilcoxon test or the t-test. The incidence of an adverse outcome (ie, graft rejection, graft loss, renal impairment, tacrolimus toxicities, and death) will be estimated using Kaplan–Meier curves. Depending on the distribution of the data, time-to-event (ie, time to reach therapeutic target range) analysis will be performed using an appropriate parametric, nonparametric, or semi-parametric survival analysis method. An ANOVA model may be utilized and tabulated along with the CIs. During the analysis, if a possible confounding variable was identified, the variable will be measured and entered into the analysis as a covariate.

Confounding factors that may impact tacrolimus response include race, age, and disease characteristics. It is possible that there may be differences in baseline demographics and disease states between the pharmacogenomic and control group. To minimize the potential impact of confounding variables on treatment response, an overall matching strategy has been incorporated into the design of the study. Please see Section 8.4 for more information regarding the matching strategy.

The exploratory endpoints will be analyzed using descriptive statistics. No formal statistical testing is planned at this time.

Statistical testing, whenever performed, will be 2-sided with a significance (alpha) level of 0.05. In addition, 95% confidence intervals will also be determined. All statistical analyses will be conducted using SAS® or other appropriate statistical programs.

8.4 Matching Strategy

As there are confounding variables, including age, race and disease state that may impact the results of the study, our study design incorporates an overall matching strategy, so that we can identify a well-matched control group. First, to control for differences in care over time, patients in the pharmacogenomic group will be matched to controls enrolled from 2010 to present. This time period was selected as there had been no major changes in standard of care or treatment regimen since 2010. After eligibility is met, control patients will be selected to match the pharmacogenomic group using a computerized matching algorithm that has been optimized to match baseline demographic and disease characteristics that have been identified a priori as likely to influence the treatment response to tacrolimus. To balance the trade-off between minimizing bias and maximizing matched sample size, a systematic approach will be conducted to identify the number of matched control patients for each patient in the pharmacogenomic group. This approach will include the following steps: 1) run the desired matching algorithm, starting with 1:1 (one control to one patient in the pharmacogenomic group) matching and iterating until the maximum desired number of potential controls per treated subject is reached; 2) for each iteration, test for covariate balance; and 3) generate numeric summaries and graphical plots of the balance statistics across all iterations in order to determine the optimal number (Linden and Samuels 2013).

The selection of patients for the control group using a matching algorithm will be conducted by an independent statistician in a blinded and unbiased manner. The statistician will have no knowledge of survival outcome, other outcome data, and genotype.

9 Safety and Adverse Events

Safety will be assessed throughout the study by evaluating physical exam, clinical chemistry, urinalysis, hematology, renal function, and incidence of adverse events (ie, graft rejection, graft loss, renal impairment, drug-related adverse events, tacrolimus toxicities, and death).

9.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others is defined as any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (ie, not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (ie, 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,
- Serious (as defined below); “serious” is different than “severe” as reported in the NCI CTCAE criteria, which applies a grade to the AE.

Adverse Event

An AE is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- results in study withdrawal
- is associated with an SAE
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

All AEs are classified as serious or nonserious. A ***serious AE*** (SAE) is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay

- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

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.

All AEs that do not meet any of the criteria for serious are regarded as ***nonserious AEs***.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

9.2 Recording of Adverse Events

AEs will be collected following kidney transplantation. AEs are recorded as part of routine standard of care for kidney transplant recipients.

At each contact with the subject, the investigator must seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs should be recorded immediately in the source document. All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine

the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

All AEs, both serious and nonserious, and deaths that occur during the patient's study participation will be recorded. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

9.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators must conform to the AE reporting timelines, formats, and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, Section 9.1).

9.3.1 Investigator reporting

For reportable deaths, the initial submission to the IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

All AEs may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of Section 9.3.

Any serious or unexpected event, which occurs to any patient in the course of their treatment on this study or within 30 days following cessation of treatment, must be reported immediately within 24 hours of the investigator learning of its occurrence. The immediate reports should be followed promptly by detailed, written reports. The Principal Investigator is responsible for reporting the SAEs to the FDA in accordance with 21 CFR 312.32 and 21 CFR 314.80. If required, an NCI Adverse Drug Reaction (ADR) form will be completed and copies sent to:

- Principal Investigator
- IRB
- UNC-CH Investigational Drug Service
- Protocol Office ADR file

For both serious and nonserious AEs, the investigator must determine both the intensity of the event and the relationship of the event to drug administration.

- Relationship to drug administration will be determined by the investigator responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the drug?
- Intensity for each AE, including any lab abnormality, will be determined by using the NCI CTCAE, version 3.0, as a guideline, wherever possible. (http://ctep.cancer.gov/forms/CTCAEv3.pdf)

9.4 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of AEs as noted above, as well as the construction and implementation of a study management plan. Please see Section 11 for more information. Medical monitoring will include a regular assessment of the number and type of SAEs.

9.5 Interim Analysis

An interim analysis will be performed on the primary endpoint when 30% of eligible patients have been enrolled and have completed the 6 months follow-up. The interim analysis will be performed by an independent statistician who is blinded for the subjects' treatment allocation.

Some of the questions that will be evaluated during the interim analysis will include:

- Is there an unexpectedly high rate of severe or life-threatening adverse events?
- Is the outcome of the trial treatment comparable with that of previous studies?

- Do the results of the interim analysis prove statistically significant differences between the trial treatments that exceed the differences defined by the statistical guidelines of the trial?

10 Data Handling and Record Keeping

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to HIPAA requirements of 1996. Those regulations require a signed subject authorization informing the subject of the following:

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

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (ie, that the subject is alive) at the end of their scheduled study period.

Of major concern to most research participants is the confidentiality of information collected as part of a research study. All information will be kept strictly confidential and used for research purposes only. No identifying information will be disclosed in reports, publications, or presentations.

Procedures to assure confidentiality will be strictly observed. The study will follow standard guidelines to assure that participant confidentiality is maintained. All data will be:

- Kept in confidential locked files;
- Identified by participant identification number (PID) only, which will be generated using random alpha numeric code only; and
- Kept separately from identifying information used for subject tracking and follow-up contacts.

The following methods will be utilized to protect the confidentiality of participant data:

1) Data collected at clinical site and entered into the database will contain a PID number that does not reflect any personal information.

2) Data linking a participant to a PID number will be stored locally in locked files. This information will not leave the clinical site and will be accessible to clinical site study staff only (but IRB may request access to patients' files during site visits).

3) Access to the data will be strictly controlled. User names and passwords will be distributed only to the appropriately trained clinical staff members.

4) Specimens collected from participants and transferred to the CGI will be coded for tracking purposes but will not contain any personal identifiers.

5) The statistician or the primary person responsible for the data analysis will not receive any original data for auditing or quality assurance purposes that contain any personal identifying information. Any personal identifiers will be completely removed prior to sending the data.

10.2 Records Retention

Methods for record retention are stated in the clinic's SOPs. Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an ICH region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

10.3 Method of Data Collection

Initially, while kidney transplant recipients receive care in the hospital, all data will be collected from medical and drug records obtained during the inpatient hospitalization. Subsequently, data on clinical, medication use, and environmental factors will be collected post-transplant in the clinic by the renal transplant coordinator who enters the patients' clinical findings, including phone conversations, in EMR.

10.4 Data Obtained for Analysis

10.4.1 Data to be collected

There will be 3 broad categories of data collected 1) patient-specific clinical and environmental data 2) genotype data, and 3) study outcomes. All participants will undergo a baseline interview to collect clinical and environmental data, and buccal swab sample will be collected for DNA extraction, genotyping, and storage for pharmacogenomic studies. At each subsequent visit, participants will undergo a follow-up interview as part of routine care to collect clinical, environmental, and outcome data.

10.4.1.1 **Clinical and Environmental Factors**

The clinical and environmental factors to be collected will be those that can alter the response to tacrolimus, increase the risk of any of the other study outcomes (eg, graft rejection), and fully describe the patient population and relevant subgroups.

Clinical factors include factors associated with altered tacrolimus dose such as age, sex, pre-existing medical conditions, and body surface area. Environmental factors include interacting medications, smoking, and alcohol use. Interacting medications will include all possible interacting medications and dietary supplements that may alter the PK or pharmacodynamics (PD) of tacrolimus. All information pertaining to clinical and environmental factors can be found in the EMR as it is routinely collected as standard care for kidney transplant recipients.

10.4.1.2 **Genetic Data**

There will be two types of genetic data: 1) genotype data related to tacrolimus dosing, which includes primary genotype data required for the dosing algorithms and 2) other genetic data that will be used for additional, ancillary analyses. The data unrelated to the dosing algorithms will be derived from the stored buccal swab sample at the Central Laboratory (CL) and are not required for the clinical trial itself. As such, they are not discussed further in this protocol. The first (genotype data required for the dosing algorithm) will be assayed at CGI under the strictest CLIA (Clinical Laboratory Improvement Amendment) standard for genotyping of human specimens. The other genotype/sequencing data will be used to define additional genetic determinants of tacrolimus dosing in patients who have disparate response from the genotype-guided dose.

Genotyping at the Central Laboratory. The CL will be responsible for: 1) extracting DNA from all participants' buccal swab samples and 2) storing DNA on all participants for future genotyping of other variants of other genes that may be important for assessing response to tacrolimus. For future studies, the CL will be responsible for developing a multiplex platform that can assay numerous genetic variants within one reaction.

Sample Collection for Storage and Analyses in Central Laboratory. Following acquisition of informed consent, buccal swab samples will be collected per manufacturer's directions. Using DNA isolation kits suitable for buccal swab samples, DNA will be isolated at the CL according to the manufacturer's instructions. A third of the aliquot will be retained by the CL, and the remainder will be delivered to CGI in a chilled insulated container. The DNA samples will be stored in barcoded tubes at the CL. All DNA samples that will be sent to CGI will also be in barcoded tubes, so that laboratory personnel at CGI are blinded to the patient's identity. All samples will be tracked using the barcode and tracking system.

10.4.2 Historical Data

Historical data from the control group will be obtained from 2010 until the end of study period. The historical controls will consist of age- and disease-matched patients who met eligibility criteria and have had previously

received kidney transplantation with standard tacrolimus dosing. These patients will also be asked to give consent for genotyping during a standard routine follow-up visit post-transplantation. Eligible patients who consented will be included in the control group and their safety and efficacy data will be collected retrospectively for up to 12 months from the initiation of the first tacrolimus dose.

10.4.3 Missing Data

The main problem from missing data will be missing tacrolimus blood levels either from attrition or missed visits. Given that the initial monitoring of tacrolimus (Day 3 and Day 7) for the primary outcome will be conducted at the hospital, we expect the issue of attrition and missing data to be very unlikely. Extensive efforts will be made to collect complete information on each subject enrolled throughout the study. The optimal approach to missing data is to be proactive and be diligent in avoiding it. Additional methods, including calendar alerts, e-mail reminders, and reminder calls may be employed to minimize missed visits.

10.5 Data Management

Using appropriate data management tools such as Research Electronic Data Capture (REDCap), the data management team will develop a data validation plan, rule set specifications, and programming logic to implement data validation rules. The rule set will include checks for missing fields, range checks, skip pattern-logic, and inter and intra form checks. Prior to analysis of the data, the data management team will perform extensive, independent testing of the validation program functionality by entering data known to violate validation rules and determining if the errors are detected.

Data validity will be monitored to address critical missing, inaccurate, illogical, or inconsistent values in the data submitted by the investigator and CL. Rules will be developed and applied to evaluate data in a hierarchical fashion: 1) safety and efficacy; 2) eligibility; 3) analysis and outcome data; and 5) descriptive non-outcome data. Rules will be implemented after the data are committed and are managed via the query tracking system.

11 Study Management

11.1 Study Monitoring Plan

This study will be monitored by the investigator. The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (eg, source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (eg, pharmacy, 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.

11.2 Central Laboratory Compliance

The CL and CGI adhere to Good Laboratory Practice (GLP) guidelines for internal consistency and accuracy of data for analyses. As the services provided by CGI and CL are highly specialized, an experienced lab investigator or external subject matter expert will be identified to accompany any laboratory visit. The following activities may occur: 1) inspection of laboratory facilities, including adequacy and security of storage space, monitoring equipment for freezer function, certification documents, hardware and software for inventory control; 2) review of current GLP, laboratory Standard Operating Procedures (SOPs), quality assurance and specimen tracking procedures; 3) review process for receiving and processing a typical specimen; 4) review quality assurance documentation and procedures that assures adherence to protocol by laboratory personnel; 5) assess methods for communication of lab specimen preparation and transfer; 6) evaluate lab procedures for maintaining records, data storage, data transmission and security; 7) examine methodology for transmission of lab data and quality control procedures regarding the data transfer process; and 8) review methods for training and documenting competency of core lab staff.

11.3 Patient Adherence with Study Medication

Compliance to tacrolimus will be assessed through measurements of tacrolimus blood levels, which are routinely done as standard of care.

11.4 Deviations from the Protocol

The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted to:

- IRB for review
- to the regulatory authority(ies), if required
- to the sponsor for agreement (if applicable)

11.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative tacrolimus dose, the study shall be conducted exactly as described in the approved protocol. Any deviation from the protocol must have prior approval by the Principal Investigator and must be recorded and explained.

11.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC-CH. The written amendment will be sent to investigators and must be submitted to the IRB. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

11.7 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP regulations and guidelines regarding clinical trials both during and after study completion.

12 Study Finances

12.1 Funding Source

This study will be funded internally through the Center for Pharmacogenomics and Individualized Therapy (CPIT).

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) will have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All UNC-CH investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

No compensation for participation will be provided. The genotyping cost will be covered by funds available through CPIT. If the patient's insurance does not cover the treatment dose (most will as it is an FDA approved dose), then we have funds available to make up the cost difference.

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ATTACHMENT 1: Adverse Event Assessment Form:

Research adverse events as of @ID@

	Adverse event (AE) history	Resolved	
	AEs reported at previous visit	YES	NO
1		☐	☐
2		☐	☐
3		☐	☐
4		☐	☐
5		☐	☐

	Since last visit, does patient have	YES	NO	Tac Dose Modification			NOTES
				Increase	Decrease	No Change	
1	Neurotoxicity (tremor, paresthesias, tingling, seizures, or headache)	☐	☐	☐	☐	☐	
2	Nephrotoxicity related to tacrolimus	☐	☐	☐	☐	☐	
3	Diarrhea, constipation	☐	☐	☐	☐	☐	
4	Peripheral edema	☐	☐	☐	☐	☐	
5	Hypertension	☐	☐	☐	☐	☐	
6	Hyperglycemia	☐	☐	☐	☐	☐	
7	Other adverse events	☐	☐	☐	☐	☐	
8		☐	☐	☐	☐	☐	