STUDY PROTOCOL

PROTOCOL TITLE:
Efficacy of achieving early target trough levels of Tacrolimus using CYP3A5 guided dosing versus weight-based dosing in a multi-ethnic population of kidney transplant recipients in Singapore

PROTOCOL NUMBER:
2019/2599

PROTOCOL VERSION: Version No. 1.3
PROTOCOL DATE: 29 September 2021

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PROTOCOL SIGNATURE PAGE

Protocol Title: Efficacy of achieving early target trough levels of Tacrolimus using CYP3A5 guided dosing versus weight-based dosing in a multi-ethnic population of kidney transplant recipients in Singapore

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Sponsor Name: SingHealth Transplant Lee Foundation Grant

Declaration of Investigator

I confirm that I have read the above-mentioned protocol and its attachments. I agree to conduct the described study in compliance with all stipulations of the protocol, regulations and ICH E6 Guideline for Good Clinical Practice (GCP).

Principal Investigator Name: Dr Ho Quan Yao

Principal Investigator Signature: 

Date: 29 September 2021

1. BACKGROUND AND RATIONALE

Tacrolimus (FK) remains the cornerstone of maintenance immunosuppressants after renal transplantation. However, it is characterised by narrow therapeutic index and large inter-individual variability in its pharmacokinetics, particularly in the dose required to reach target trough blood concentrations. Among several factors investigated for their possible influence on tacrolimus pharmacokinetics, polymorphisms in genes coding for biotransformation enzymes (cytochrome P450 (CYP) isoenzymes 3A4 and 3A5) have received much attention. Exposure to FK correlates with the cytochrome P450 (CYP) 3A4 and CYP3A5 which are polymorphically expressed. This is in part explained by the presence of single-nucleotide polymorphisms (SNPs) in the CYP3A5 and CYP3A4 genes.

A SNP at position 6986 of the CYP3A5 gene (rs776746; 6986A>G; designated as CYP3A5 *3) results in a splicing defect that causes non-functional CYP3A5 protein (Birdwell KA et al, 2015). In retrospective studies, it has been demonstrated that whole-blood tacrolimus trough concentration and tacrolimus dose requirements are closely associated with CYP3A5 polymorphism: patients carrying at least one CYP3A5*1 allele have a lower tacrolimus concentration/dose ratio than that in nonexpressers (CYP3A5*3/*3) among kidney, liver, lung, and heart transplant recipients. In a cohort of kidney transplant recipients, a 2.3-fold difference was observed in the daily dose required to maintain target FK trough concentration in CYP3A5*3*3 and CYP3A5*1*1 patients (Haufroid V et al, 2004). Moreover, a significant delay in reaching target FK trough cocentration and a shorter time to acute rejection were observed during the first 2 weeks after kidney transplantation in CYP3A5 expressers (MacPhee et al, 2004).

To date, renal transplant (RTx) recipients receive standard weight-based dosing of FK and therapeutic drug monitoring is employed for subsequent dose adjustment to ensure target FK concentration is attained. However, the current weight-based dosing strategies to guide the initial FK dosing have been poorly predictive of the actual FK dose required to attain therapeutic FK level (Loh PT et al, 2008). With the increased possibility of sub-therapeutic FK level during the early phase post renal transplantation, it puts them at a higher risk of developing acute rejection.

There has been increasing evidence to suggest the implementation of pre-transplantation genotyping to guide the initial FK dose to achieve target FK concentrations as quickly as possible (Chen SY et al, 2013; Shuker N et al, 2016; Pallen N et al, 2016; Zhang J et al. 2010). Min SI et al revealed that in RTx patients who received equal doses of FK, the CYP3A5 expressers (*1*1 and *1*3 genotypes) had lower FK level and a higher incidence of acute cellular mediated rejection than the CYP3A5 non-expressers (CYP35 *3*3 genotype). Furthermore, multivariate analysis identified CYP3A5 expression as an independent risk factor for cellular mediated rejection (odds ratio 2.79; p=0.043) (Min SI et al, 2010). On the contrary, there are a few studies that report contradictory results of genotype-guided FK dosing as being useful in attainment of target therapeutic levels. A randomized controlled trial by Thervet et al demonstrated that while target FK levels were achieved earlier in incident kidney transplant recipients whose tacrolimus dose was chosen based on CYP3A5 genotype versus a control group that started tacrolimus based on standard weight-based dosing (Thervet et al, 2010), no

differences were seen in patient survival, nephrotoxicity, or acute rejection over the three-month follow-up. A key limitation of the study was tacrolimus was introduced only 7 days after transplantation in the CYP3A5 genotype-based dosing group while it was started at time of transplantation in the weight-based dosing group. Prevalence of CYP3A5 genotypes is also different in various ethnicities.For example, the Caucasian proportions of CYP3A5 expressers (CYP3A5 *1*1 and *1*3) were 18% while in our local multiethnic population, CYP3A5 expressers make up 51% of the renal transplant population (Loh PT et al, 2008).

Given the differences in CYP3A5 genotype prevalence among races and the controversy in clinical benefits of such a pro-active dosage strategy, the impact of CYP3A5 genotype-guided dosing on clinical outcome remains to be answered, especially in the local multi-ethnic population. This pro-active approach may also sound promising for the local multi-ethnic population where majority of the renal transplant population are CYP3A5 expressers who may require a higher initial dose of FK based on genotyping profile (Loh PT et al, 2008). Faster achievement of target concentrations could potentially reduce the risk of graft rejection because of underexposure and toxicity because of overexposure (Birdwell KA et al, 2015). A recent meta-analysis including 21 studies evaluating the effect of CYP3A5 polymorphism on kidney transplant recipients concluded that there is a significantly increased risk for transplant rejection for those with the CYP3A5*1*1 or CYP3A5*1*3 genotype (P=0.04; odds ratio=1.32) (Rojas et al, 2015). Furthermore, patients with the CYP3A5*3*3 (nonexpresser) genotype exhibited dose-adjusted trough concentrations 1.8–2.5 times higher than CYP3A5 expressers during the first year after transplantation (Rojas et al, 2015).

This study aims to shed light on the possible impact of CYP3A genotype-based FK dosing on FK target achievement and clinical outcome after RTx in a multi-ethnic population where current evidence is lacking. This data would be helpful to the physicians so that by knowing the genotype of the patient before undergoing transplantation, they would be able to decide on the starting dose of FK so as to avoid low trough levels and risk of acute rejection or high trough levels and risk of nephrotoxicity.

2. HYPOTHESIS AND OBJECTIVES

We hypothesise that the adaptation of CYP3A5 genotype-based FK dosing will lead to earlier FK target achievement and consequently, better clinical outcome after RTx.

This study aims to shed light on the possible impact of CYP3A genotype-based FK dosing on FK target achievement and clinical outcome after RTx in a multi-ethnic population where current evidence is lacking. This data would be helpful to the physicians so that by knowing the genotype of the patient before undergoing transplantation, they would be able to decide on the starting dose of FK so as to avoid low trough levels and risk of acute rejection or high trough levels and risk of nephrotoxicity.

3. EXPECTED RIKS AND BENEFITS

The expected risks to the subjects include:

- Risks of venipuncture – Two additional blood tubes containing 3mls (1 teaspoon) of blood will be collected which will be timed with the other regular blood tests to minimize the discomfort of venipuncture

- Breach of patient confidentiality - Personal data will be collected and stored in a secured, password encrypted device which will only be accessed by the investigators to maintain patient confidentiality
- Risks of supra- or subtherapeutic tacrolimus levels - There could be potential risk associated with high tacrolimus dose/level (e.g. risk of high potassium, liver impairment, renal impairment) and low tacrolimus dose/level (e.g. risk of kidney rejection). This may potentially occur regardless of whether the subjects are dosed by genotyping or by standard weight-based dosing

The expected benefits to the subjects include:

- There is no assurance that the subject will directly benefit from this study. However, their participation may contribute to the medical knowledge about the potential use of identifying various CYP3A5 gene version to determine the initial dosing of tacrolimus in kidney transplant recipients

4. STUDY POPULATION

4.1. List the number and nature of subjects to be enrolled.

The number of living kidney donor transplants performed at Singapore General Hospital (SGH) is low - usually between 20 to 30 kidney transplants per year. Therefore, it is unrealistic to plan for a randomized controlled study as it will take years to achieve sufficient numbers for an adequately powered study. We aim for this study to be a pilot study to determine if there is any trend in correlation between CYP3A4 genotype and dosing. If there is any trend in correlation, we can then propose to design an adequately powered and likely multi-centre study to confirm any preliminary findings from this pilot study.

Thence it is decided that we will aim to recruit 100 subjects who are between the ages of 21 and 75 years old and are scheduled to receive renal transplant from a living donor and follow up at SGH between January 2021 to January 2023. The control group selected will be a historical cohort which had received tacrolimus based on weight-based dosing while the intervention group will be a prospective cohort.

4.2. Criteria for Recruitment and Recruitment Process

For the historical control group, all patients who have received living donor renal transplant between January 2016 to December 2020 at Singapore General Hospital and still currently on follow-up will be screened by the investigators to see if they meet the eligibility criteria and recruited if all criteria are met.

For the prospective group, all patients planned for a living donor renal transplant at Singapore General Hospital between January 2021 to January 2023 will be identified by the transplant coordinators and screened by the investigators to see if the subject meet the eligibility criteria and recruited if all criteria are met.

4.3. Inclusion Criteria

To be eligible for inclusion into the study, the subject should meet the following criteria:

- On follow up at SGH between the ages of 21 and 75 years old who had received or are

scheduled to receive a living donor renal transplant between January 2016 to January 2023

- Has to receive tacrolimus (FK) (Prograf®; Astellas Pharma, Singapore), mycophenolic acid (MPA) (Cellcept®; Roche, Basel, Switzerland or Myfortic®; Novartis Pharma AG, Basel, Switzerland) and prednisolone as triple immunosuppressive drug maintenance regimen

4.4. Exclusion Criteria

A subject will be excluded from the study if any of the following criteria are met:

- Planned to be initiated on non-standard doses of tacrolimus (e.g. planned to initiate on sub-therapeutic doses of tacrolimus
- Evidence of active liver disease or gastrointestinal disorder that might interfere with the ability to absorb oral medication
- Contraindications to tacrolimus (FK) – e.g. hypersensitivity
- Takes concurrent medications which are known to severely interact with FK (e.g. verapamil, azoles, rifampicin, erythromycin or clarithromycin

5. STUDY DESIGN AND PROCEDURES/METHODOLOGY

Methodology

This is a prospective, investigator-initiated, open-label single center pilot study conducted at the Singapore General Hospital (SGH). It will compare selected end-points between a historical control group (which was dosed by mg/kg) and a prospective intervention group (which will be dosed according to genotype).

Treatment/dosing:

The standard arm will comprise of historical controls who are patients who had received renal transplant from a living donor and followed up at SGH between January 2016 - December 2020 and received standard weight-based dosing of tacrolimus.

The intervention genotyping arm will comprise of patients who are scheduled to receive renal transplant from a living donor between January 2021 to January 2023. They will be assigned to receive the initial CYP3A5 genotype-based tacrolimus (FK) dose as determined by their CYP3A5 genotype:

Likely phenotype	Diplotypes	Initial Prograf® dosing (mg/kg/day given in 2 divided doses)
Extensive metabolizer (CYP3A5 expresser)	CYP3A*1*1	0.20
Intermediate metabolizer (CYP3A5 expresser)	CYP3A*1*3	
Poor metabolizer (CYP3A5 non-expresser)	CYP3A*3*3	0.15

The FK dose will be rounded off to the nearest 0.5 mg. FK will be administered at 8 am and 8 pm on an empty stomach.

Rationale for Selection of Dose:

Patients will be assigned to receive an initial CYP3A5 genotype-based tacrolimus (FK) dose of

0.15 mg/kg/day (CYP3A5 non-expresser) or 0.20 mg/kg/day (CYP3A5 expresser).

The current Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 genotype and Tacrolimus Dosing recommend that

- CYP3A5 expressers e.g. carriers of at least one CYP3A5*1 allele) receive 1.5 – 2 times the standard starting dose of tacrolimus
- CYP3A5 non-expressers e.g. carriers of CYP3A5 *3/*3 receive 0.075 mg/kg/dose twice daily

At SGH, the standard starting dose of Prograf® is 0.15 mg/kg/day. Based on the CPIC guidelines, CYP3A5 expressers will receive an initial dose of 0.20 mg/kg/day (rounded down). Subsequent drug doses will be adjusted based on drug levels.

All the proposed initial doses of tacrolimus are within the local approved package insert for Prograf®, which recommends an initial dose of 0.15 to 0.30 mg/kg/day for adult kidney transplant patients.

Induction therapy

All patients will receive induction agents with basiliximab (Simulect®; Novartis Pharma B.V., Arnhem, The Netherlands) which is given as a 20 mg dose intravenously before graft reperfusion, followed by a second infusion of 20 mg on day 4 post transplantation, or intravenous antithymocyte globulin (Thymoglobulin®; Genzyme, Cambridge, MA) at an initial dosage of 1-1.5 mg/kg/day for 3 - 7 days. Two doses of 500 mg methylprednisolone will be given intravenously on day 0 and day 1 after transplantation

Maintenance therapy

Patients will receive a starting oral dose of mycophenolate mofetil 1 g twice daily (MMF; Cellcept®; Roche, Basel, Switzerland) or equivalent dose of mycophenolate acid EC 720 mg twice daily (MYF; Myfortic®; Novartis Pharma AG, Basel, Switzerland) based on centre practice. Steroid therapy will be initiated on day 2 with oral prednisolone 20 mg once daily for the first week, followed by weekly reduction of 2 mg until a nadir of 5 mg daily is attained. The taper schedule and corticosteroid dose may be adjusted at the discretion of the investigator (e.g. if biopsy-proven acute rejection occurs).

Prophylaxis against infection

All patients receive sulfamethoxazole/trimethoprim, pentamidine inhalation or other alternatives (if sulfamethoxazole/trimethoprim is contra-indicated) for Pneumocystis jirovecii pneumonia prophylaxis. Cytomegalovirus (CMV) prophylaxis with intravenous ganciclovir or oral valganciclovir will be initiated within the first 10 days post transplantation, with the duration of CMV prophylaxis (either 3 months or 6 months) depending on the donor and recipient CMV serostatus and whether antithymocyte globulin is given. Antifungal prophylaxis with nystatin will be given for 1 month post-transplant.

Endpoints

The primary endpoint of the study is the proportion of patients within the desired FK trough level at first steady state e.g. morning of day 3 after five unaltered FK doses and the proportion of patients within target FK levels on day 7 post initiation of FK. The FK trough level is defined as the whole blood concentration 12 h ± 15 min after the previous dose taken the night before at 8pm. Thereafter, the clinicians can change the FK dose based on the measured FK

level and/or the clinical situation of the individual patient according to routine transplant immunosuppression protocol. For the genotyping arm, the pre-specified FK target range will be 10 – 15 ng/mL in the first 1 week after transplantation and subsequently adjusted based on routine transplant immunosuppression protocol.

Secondary pharmacokinetic endpoints include

- Proportion of patients within target FK levels on day 7 post renal transplant
- Average FK level during the first 1 weeks after transplantation
- Average daily dose of FK the first 90 days post transplantation [days 3, 7, 14±3, 30±3, 60±3, 90±3 days post transplantation]
- Average concentration-to-dose ratio of FK during the first 90 days post transplantation [days 3, 7, 14±3, 30±3, 60+±3, 90±3 days post transplantation]
- Time to reach the first target FK range
- Number of FK dose adjustments required to reach the target FK level
- Number of markedly sub-therapeutic FK level (defined as <4 ng/mL) and markedly supra-therapeutic FK level (defined as > 20 ng/mL)

Secondary clinical endpoints include

- Incidence of biopsy-proven acute rejection (BPAR), with histologic characteristics described accordingly to the Banff criteria [Banff 1997 scoring system with 2007 modifications] and/or clinically presumed acute rejection
- Incidence of graft loss (defined as failure to discontinue dialysis or if patient undergoes graft nephrectomy)
- Renal function at month 3 after transplantation. Estimated GFR (eGFR) will be calculated using CKDEPI equation. [Time Frame: at day 7, 14, 30±3, 60±3 and 90±3 days after transplantation]

All patients will be followed for at least 3 months after transplantation, or until graft loss occurs (defined as death with a functioning transplant, return to dialysis or re-transplantation), whichever occurs earlier.

Safety

The frequencies of serious adverse events which included death, graft loss, infections (including CMV and BK infection), post-transplant diabetes mellitus, neurologic events, transaminitis and acute FK-induced nephrotoxicity will be recorded. Acute FK toxicity was defined as ≥ 15% increase in serum creatinine with a return to baseline after FK dose reduction and after exclusion of other causes of renal transplant graft function deterioration.

FK measurement and genotyping

FK trough level will be determined in whole blood using liquid chromatography and tandem mass spectrometry (LC-MS/MS) method.

Two venous blood samples (3 mL each) for CYP3A5 genotyping will be obtained from all study participants. The subjects' blood samples will be de-identified and sent to the molecular laboratory in Tan Tock Seng Hospital, Singapore, which is offering CYP3A5 genotyping as a clinical service. Briefly, the genomic DNA is extracted from ethylenediaminetetraacetic acid anticoagulated whole blood samples using a QIAamp DNA mini kit (Qiagen, Hilden,

Germany), according to the manufacturer's protocol. Real-time polymerase chain reaction (RTPCR) is then performed with a 7900 Real-Time PCR System. RT-PCR consisted of a denaturation step at 95ºC for 10 minutes, then 50 cycles of 15 seconds at 92ºC and 1 minute 30 seconds at 69ºC. CYP3A5 6986A>G (rs776746) allelic discrimination is undertaken with a Custom TaqMan® SNP Genotyping Assay and VIC and FAM reporter dyes. No phenotyping will be done for CYP3A5. Once the blood sample has been genotyped, it will be destroyed as agreed upon by the molecular laboratory at Tan Tock Seng Hospital. The genotyping results will then be re-identified by the PI or co-investigators. The genotyping result will not be communicated to the subjects.

The blood sample will be destroyed immediately after sample analysis.

Clinical assessment

Demographic and baseline data included the age, gender, race, weight and height of the recipient, cold ischemia time, the flow panel-reactive antibody value before transplantation and at its peak, the CMV serostatus of the donor and recipient. Laboratory data — including hemoglobin level, white-cell and platelet counts, FK trough level, liver function panel, serum creatinine level and estimated glomerular filtration rate — are measured at baseline, days 0 through 7,14±3, 30±3, 60±3 and 90±3.

Withdrawal from study

Subjects may withdraw voluntarily from participation in the study at any time.
Possible reasons for discontinuation from the study include

- Switched out from tacrolimus to other immunosuppressants
- No longer on triple immunosuppressants either due to intolerance or infection-related complications
- Graft failure or death

If voluntary withdrawal occurs, the subject will be asked to continue scheduled evaluations, complete an end of study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the subject's condition becomes stable.

Research Data Confidentiality

Data will be retrieved from the time of preparation for renal transplantation to 3 months after renal transplantation. Any individually-identifiable data obtained during the course of this study will be stored and used only for the purposes of this study. These data will not be used for future research.

The study team will store all research data in a password protected computer in the investigators' office with key access. Only the principal investigator and co-investigators have access to the research data and the research data will be saved in a password protected file.
The research data will be retained in a secured storage facility for a minimum of 7 years after completion of research study or date of publication of the research using the research data, whichever is later. These documents will be retained by the Principal Investigator in a secure storage facility, accessible for inspection and copying by authorized authorities.

6. SAFETY MEASUREMENTS

6.1. Definitions

Serious adverse event (SAE) in relation to human biomedical research, means any untoward medical occurrence as a result of any human biomedical research which:

- results in or contributes to death
- is life-threatening
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in or contributes to persistent or significant disability/incapacity or
- results in or contributes to a congenital anomaly/birth defect
- results in such other events as may be prescribed

Adverse event (AE) in relation to human biomedical research means any untoward medical occurrence as a result of any human biomedical research which is NOT serious. Adverse event can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease possibly/ probably/ definitely associated with the participant in the human biomedical research.

6.2. Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to CIRB

Only related SAEs (definitely/ probably/ possibly) will be reported to CIRB. Related means there is a reasonable possibility that the event may have been caused by participation in the research. Please refer to the CIRB website for more information on Reporting Requirement and Timeline for Serious Adverse Events.

The investigator is responsible for informing CIRB after first knowledge that the case qualifies for reporting. Follow-up information will be actively sought and submitted as it becomes available.

Related AEs will not be reported to CIRB. However, the investigator is responsible to keep record of such AEs cases at the Study Site File.

6.3. Safety Monitoring Plan

The data and safety monitoring will be performed by the Principal Investigator (PI) and a team of Co-Investigators. The patient will be reviewed daily when hospitalized and will then be followed up at outpatient as per current standard of care. Data will be reviewed by the PI on a three-monthly basis and it will be stored in an encrypted device. Study will be stopped if more than 25% of the patients in the genotyping arm have a tacrolimus trough of < 3 ng/mL or > 20 ng/mL on day 3

As an additional safety precaution, the tacrolimus drug levels achieved with current protocol and the starting dose of tacrolimus will be reviewed after every 10 participants recruited. The starting dose will not deviate from the dosage recommended in the drug insert.

6.4. Complaint Handling

Complaints from research participants will be addressed by the principal investigators or co-investigators to ensure that suitable resolutions can be identified to protect the rights and welfare of research participants, while ensuring appropriate privacy and confidentiality protections are in place throughout the process. Research participants may also contact the SingHealth Centralised Institutional Review Board for any feedback about the research study.

7. DATA ANALYSIS

7.1. Data Quality Assurance

Data will be collected by investigators who are trained in the field of renal transplantation to ensure that the data is accurate, complete and reliable. For subjects in the prospective arm, the data will also be collected real-time to ensure that the information is utilized efficiently.

Data will be reviewed by the PI on a three-monthly basis. After data collection, 10% of cases will be randomly selected for review to ensure accurate and complete data collection, with an acceptable error rate of <5%

7.2. Data Entry and Storage

Data will be retrieved from the time of preparation for renal transplantation to 3 months after renal transplantation and stored electronically in a password protected file. Any individually-identifiable data obtained during the course of this study will be stored and used only for the purposes of this study. These data will not be used for future research.

The study team will store all research data in a password protected computer in the investigators' office with key access. Only the principal investigator and co-investigators have access to the research data and the research data will be saved in a password protected file.
The research data will be retained in a secured storage facility for a minimum of 7 years after completion of research study or date of publication of the research using the research data, whichever is later. These documents will be retained by the Principal Investigator in a secure storage facility, accessible for inspection and copying by authorized authorities.

8. SAMPLE SIZE AND STATISTICAL METHODS

8.1. Determination of Sample Size

The number of living kidney donor transplants performed at Singapore General Hospital (SGH) is low - usually between 10 to 20 kidney transplants per year. Therefore, it is unrealistic to plan for a randomized controlled study as it will take years to achieve sufficient numbers for an adequately powered study. We aim for this study to be a pilot study to determine if there is any trend in correlation between CYP3A4 genotype and dosing. If there is any trend in correlation, we can then propose to design an adequately powered and likely multi-centre study to confirm any preliminary findings from this pilot study

Thence, upon consultation with the biostatistician, it is decided that we will recruit 100 subjects who are between the ages of 21 and 75 years old and are scheduled to receive renal transplant

from a living donor and follow up at SGH between January 2016 to January 2023. The control group selected will be a historical cohort which had received tacrolimus based on weight-based dosing while the intervention group will be a prospective cohort.

8.2. Statistical and Analytical Plans

For the analysis of efficacy, the intention-to-treat analysis will be used, which will include all patients who were treated with at least one dose of FK. Categorical variables are reported using frequency and percentages while continuous variables are expressed as medians with ranges. A chi-square statistic will be used to evaluate the null hypothesis of no difference between the standard dose group and genotype based group within the FK target range on day 3 after transplantation. Continuous data will be compared using the Mann-Whitney U test. The time to reach target FK range will be estimated with Kaplan-Meier survival analysis and compared between the groups using log-rank test. To estimate the overall effect of CYP3A5 genotype on the outcome variables of FK daily dose, FK trough concentration and dose-adjusted FK level (a measure of apparent oral clearance), a mixed between-within subjects analysis of variance will be used. This analysis will also be used to estimate the overall effect of dosing approach on renal function. All data are analysed using R Core Team (2017). A p-value < 0.05 is considered statistically significant.

9. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator(s)/institution(s) will permit study-related monitoring, audits and/or IRB review and regulatory inspection(s), providing direct access to source data/document.

10. QUALITY CONTROL AND QUALITY ASSURANCE

Data will be reviewed by the principal investigator and co-investigators at 3-monthly intervals to ensure for adherence with the protocol and for accuracy in relation to source documents.

11. ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the Good Clinical Practice and the applicable regulatory requirements.

This final Study Protocol, including the final version of the Participant Information and Consent Form, must be approved in writing by the Centralised Institutional Review Board (CIRB), prior to enrolment of any patient into the study.

The principal investigator is responsible for informing the CIRB of any amendments to the protocol or other study-related documents, as per local requirement.

11.1. Informed Consent

Participants will be approached during their routine clinic visit before transplant ethic

committee by the investigators to participate in the study and the informed consent will be taken in the presence of a witness. The consent process will take place in a consultation room in the clinic to ensure privacy and allow the investigator to explain the nature of the study to the participants. The consent process informs the participant about the study, indicates the participation is voluntary and he/she has the right to stop at any time. Risks are enumerated in the informed consent form and described orally during the consent process. The consent will be communicated in a language that is understood by the prospective participant or the legal representative. Participants will be given ample time during the clinic visit to consider participation in study. If the potential research participant does not speak or understand English, a translator will be engaged to help in the consent taking process and will act as witness for the informed consent. Copies of the current, IRB-approved consent form detailing the risks and benefits of study participation will be provided to the participants.

In obtaining and documenting informed consent, the investigator will comply with the GCP guidelines and to the ethical principles that have their origin in the Declaration of Helsinki.

11.2. Confidentiality of Data and Patient Records

Blood samples that are sent to Tan Tock Seng Hospital, Singapore, will be de-identified and destroyed immediately after sample analysis. The genotyping result will then be re-coded back by the investigators.

The study team will store all research data in a password protected computer in the investigators' office with key access. Only the principal investigator and co-investigators have access to the research data and the research data will be saved in a password protected file.
The research data will be retained in a secured storage facility for a minimum of 7 years after completion of research study or date of publication of the research using the research data, whichever is later. These documents will be retained by the Principal Investigator in a secure storage facility, accessible for inspection and copying by authorized authorities.

12. PUBLICATIONS

Publication including the assignment of authorship will be based on the International Committee of Medical Journal Editors (ICMJE) Recommendations. Individuals who do not meet the recommended requirements for authorship, but have provided a valuable contribution to the work, will be acknowledged for their contributing role as appropriate to the publication.

13. RETENTION OF STUDY DOCUMENTS

The research data will be retained in a secured storage facility for a minimum of 7 years after completion of research study or date of publication of the research using the research data, whichever is later. These documents will be retained by the Principal Investigator in a secure storage facility, accessible for inspection and copying by authorized authorities.

14. FUNDING and INSURANCE

The study is funded by SingHealth Transplant Lee Foundation Grant which has provided a total sum of $25,000 starting from 4 January 2021 till 3 January 2023.

List of Attachments

Appendix 1 *References*

Appendix 2 **Principal Investigator's CV**

Appendix 3 *IRB Approval Letter*

Appendix 4 *Data Collection Form*

Appendix 5 *Informed Consent Forms*

Appendix 1 – References

1. Chen SY et al. Individualization of tacrolimus dosage basing on cytochrome P450 3A5 polymorphism--a prospective, randomized, controlled study.Clin Transplant. 2013 May-Jun;27(3):E272-81.
2. Shuker N et al. A Randomized Controlled Trial Comparing the Efficacy of Cyp3a5 Genotype-Based With Body-Weight-Based Tacrolimus Dosing After Living Donor Kidney Transplantation. A Randomized Controlled Trial Comparing the Efficacy of Cyp3a5 Genotype-Based With Body-Weight-Based Tacrolimus Dosing After Living Donor Kidney Transplantation. Am J Transplant. 2016 Jul;16(7):2085-96.
3. Birdwell KA et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing.Clin Pharmacol Ther. 2015 Jul;98(1):19-24.
4. Pallet N et al. Long-Term Clinical Impact of Adaptation of Initial Tacrolimus Dosing to CYP3A5 Genotype. Am J Transplant. 2016 Sep;16(9):2670-5.
5. Zhang J et al. Value of CYP3A5 genotyping on determining initial dosages of tacrolimus for Chinese renal transplant recipients. Transplant Proc. 2010 Nov;42(9):3459-64.
6. Loh PT et al. Significant impact of gene polymorphisms on tacrolimus but not cyclosporine dosing in Asian renal transplant recipients. Transplantation proceedings. 2008;40(5):1690-1695
7. Thervet et al. Optimization of Initial Tacrolimus Dose Using Pharmacogenetic Testing. Clin. Pharmacol Ther. 2010; 17: 2010
8. MacPhee et al. The Influence of Pharmacogenetics on the Time to Achieve Target Tacrolimus Concentrations after Kidney Transplantation. Am J Transplant. 2004; 4(6): 914-9
9. Min SI et al. CYP3A5*1 Allele: Impacts on Early Acute Rejection and Graft Function in Tacrolimus-Based Renal Transplant Recipients. Transplantation. 2010;90(12):1394-1400.

Appendix 2 – Principal Investigator's CV

Curriculum Vitae of Dr Ho Quan Yao

Personal Particulars:

Salutation: Dr
Name of Co-I: Ho Quan Yao
Designation: Consultant
Department/ACP/Institution: Renal Medicine / Medicine / Singapore General Hospital
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Professional Experience:

Current Position(s): Consultant
Previous Appointments: Resident, Senior Resident, Associate Consultant
Academic Qualifications

Degree/year	*Institution*	*Discipline*
Certificate of Specialist Accreditation 2018	*Specialist Accreditation Board Ministry of Health, Singapore*	*Nephrology*
MRCP / 2014	*Royal College of Physicians* United Kingdom	*Internal Medicine*
MMed / 2014	*YLLSoM, National University of Singapore*	*Medicine*
ECFMG / 2010	*Education Commission for Foreign Medical Graduates*	*Medicine*
MBBS / 2009	*YLLSoM, National University of Singapore*	*Medicine and Surgery*

Current Research Activities

1. COVID-19 in kidney transplant recipients – a systematic review and meta-analysis
2. Kidney biopsy in kidney transplant recipients – a systematic review and meta-analysis
3. Retrospective studies and reviews on post-transplantation outcomes including outcomes after HLA or ABO incompatible kidney transplantation, waitlist criteria review, post-transplant malignancy, pre- and post-transplant cardiovascular disease
4. Extracorporeal therapies in renal transplantation – therapeutic plasma exchange, double-filtration plasmapheresis (DFPP)
5. Vascular access related complications in kidney transplant recipients

List of Invited Talks (in the last 2 years)

1. Singapore Urological Association (SUA) Campbell Book Club 2020 / Renal Pathophysiology (Fluid & Electrolytes) In Critical Care, 29 August 2020

Grants Awarded (International, National or Internal sources):

Project Title	Origin	Name of Grant	Funding Agency	Funding Amount ($)	Project Date
Efficacy of achieving early target trough levels of Tacrolimus using CYP3A5 guided dosing versus weight-based dosing in a multi-ethnic population of kidney transplant recipients in Singapore	Investigator-initiated	SingHealth Duke-NUS Transplant Centre Lee Foundation Grant	Lee Foundation	$25,000	4 January 2021 till 3 January 2023

Awards and Honors (selective) :

1. Outstanding Faculty Award in Residency in SingHealth Excels (RiSE) Awards 2019
2. Service with a Heart Award 2019
3. NHG Teaching Award for Junior Clinicians 2016
4. Resident of the Year Award, National Healthcare Group (NHG) IM Residency Program 2011
5. PGY1 Undergraduate Teaching Merit Award, Tan Tock Seng Hospital 2011
6. Infectious Disease Book Prize, YLLSoM, NUS 2009

Patents: Nil

Publications (selective):

1. Mahaboob S, Lim LK, Ng CL, Ho QY, Leow ME, Lim EC. Developing the "NUS Tummy Dummy", a low-cost simulator to teach medical students to perform the abdominal examination. Ann Acad Med Singap. 2010 Feb;39(2):150-1. PMID: 20237739.

2. Ho QY, Tan CS, Thien SY, Kee T, Chlebicki MP. The use of intravesical cidofovir for the treatment of adenovirus-associated haemorrhagic cystitis in a kidney transplant recipient. Clin Kidney J. 2019 Feb 18;12(5):745-747. doi: 10.1093/ckj/sfz016. PMID: 31583099; PMCID: PMC6768302.

3. Ho QY, Lim CC, Thangaraju S, Siow B, Chin YM, Hao Y, Lee PH, Foo M, Tan CS, Kee T. Bleeding Complications and Adverse Events After Desmopressin Acetate for Percutaneous Renal Transplant Biopsy. Ann Acad Med Singap. 2020 Feb;49(2):52-64. PMID: 32246706.

4. Ho QY, Chung SJ, Gan VHL, Ng LG, Tan BH, Kee TYS. High-immunological risk living donor renal transplant during the COVID-19 outbreak: Uncertainties and ethical dilemmas. Am J Transplant. 2020 Jul;20(7):1949-1951. doi: 10.1111/ajt.15949. Epub 2020 May 8. PMID: 32337825; PMCID: PMC7267290.

5. Ho QY, Chung SJ, Low SCS, Chen RC, Teh SP, Chan FZY, Tan BH, Kee TYS. Evaluating Potential Deceased Donor Renal Transplant Recipients for Asymptomatic COVID-19. Transplant Direct. 2020 May 22;6(6):e559. doi: 10.1097/TXD.0000000000001010. PMID: 32607425; PMCID: PMC7266364.

6. Tan MSH, Chung SJ, Ho QY, Thangaraju S, Kee TYS. A single-centre observational study comparing the impact of different cytomegalovirus prophylaxis strategies on cytomegalovirus infections in kidney transplant recipients. Proceedings of Singapore Healthcare. September 2020. doi:10.1177/2010105820953461.

7. Ong PW, Kee T, Ho QY. Impact of tacrolimus versus cyclosporine on one-year renal transplant outcomes: A single-centre retrospective cohort study. Proceedings of Singapore Healthcare. September 2020. doi:10.1177/2010105820957370.

8. Yap YT, Ho QY, Kee T, Ng CY, Chionh CY. The Impact of Pre-Transplant Biopsy on 5-Year Outcomes of Expanded Criteria Donor Kidney Transplantation. Nephrology (Carlton). 2020 Sep 28. doi: 10.1111/nep.13788. Epub ahead of print. PMID: 32986301.

9. Kee T, Gan V, Chung JS, Tee PS, Lu YM, Chan LP, Cheong E, Lee PH, Yong JH, Ho QY, Thanagaraju S, Foo F, Kwan N, Ng E, Xia H, Lee C, Boey S, Foo M, Tan CS. Managing a renal transplant program during the Coronavirus Disease 2019 (COVID-19) Pandemic: Practical experience from a Singapore Transplant Centre. Ann Acad Med Singap. 2020;49:652–60

10. Goh LH, Wong J, Chng TW, Chew MH, Kee T, Ho QY. Extra-intestinal sodium polystyrene sulfonate crystal-induced inflammatory pseudotumour in an asymptomatic haemodialysis patient [published online ahead of print, 2021 Jan 3]. Int Urol Nephrol. 2021;10.1007/s11255-020-02702-9. doi:10.1007/s11255-020-02702-9. PMID: 33389507.

11. Liew ZH, Tan PH, Foo M, Kee T, Ho QY. Nodular glomerulosclerosis in a kidney transplant recipient with impaired glucose tolerance: diabetic or idiopathic? A case report and literature review [published online ahead of print, 2021 Jan 3]. CEN Case Rep. 2021;10.1007/s13730-020-00546-x. doi:10.1007/s13730-020-00546-x. PMID: 33393072.

12. SW Tan, J Wong, T Kee, ZT Chia, QY Ho, MMF Chan, KY Chew, CC Oh. Successful Treatment of Calciphylaxis in A Renal Transplant Recipient with combination of Intralesional Sodium Thiosulphate, Intravenous Sodium Thiosulphate, and Fish Skin Graft. Australas J Dermatol. 2020. Manuscript accepted for publication.

Other Duties (selective):

1. Coordinator for SingHealth Renal Medicine Residency Programme in Transplant Curriculum
2. Physician Core Faculty Member SingHealth Renal Medicine Residency Programme
3. Physician lead, Deceased Donor Kidney Transplant Program