Preservation of renal function after liver transplant for patients with pre-existing chronic kidney disease or peri-operative acute kidney injury using Everolimus plus mycophenolic acid immunosuppression regimen.

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1.0 Background

Tacrolimus is the standard immunosuppressive drug used to prevent organ rejection post liver transplant. One side effect of Tacrolimus is nephrotoxicity. Everolimus does not have the nephrotoxicity side effects of Tacrolimus. Replacement of Tacrolimus by Everolimus may have a reduced incidence of renal dysfunction in liver transplant patients who already have chronic kidney disease or peri-operative acute kidney injury. Our liver transplant patients receive potent induction immunosuppression in the form of rabbit anti thymocyte globulin. We believe that in conjunction with this induction regimen, patients can be maintained on Everolimus monotherapy without the risk of rejection. Additionally, Everolimus is known to induce tolerance in transplant recipients. Tolerant patients do not require immunosuppression to accept transplant organs.

2.0 Rationale and Specific Aims

Tacrolimus is a widely used in liver transplant recipients for immunosuppression, however it is associated with nephrotoxicity. Everolimus, on the other hand lacks nephrotoxicity. Whether replacement of tacrolimus by Everolimus preserves kidney function in patients with pre-existing chronic kidney disease or acute kidney injury is not well established. Also, the efficacy and safety of reduced-dose Everolimus with or without mycophenolic acid (Cellcept or Myfortic) in prevention of rejection is unknown.

Primary Aim

Assess the effect of Everolimus with or without mycophenolic acid versus Tacrolimus plus mycophenolic acid therapy on renal function measured by Glomerular Filtration Rate (GFR).

Secondary Aims

Compare the efficacy of Everolimus plus mycophenolic acid versus Tacrolimus plus mycophenolic acid therapy as measured by the following:

- Biopsy-confirmed acute rejection
- Hyperlipidemia
- Proteinuria

- NODAT [New Onset Diabetes mellitus After Transplant], hypertension and malignancy

3.0 Study Plan, Experimental Design, and Methodology

The proposed investigation will be a single-center, randomized, open-label, active control comparison of the standard renal dysfunction maintenance immunosuppression regimen of reduced-dose Tacrolimus plus mycophenolic acid (control arm) versus Everolimus in combination with mycophenolic acid (study arm).

4.0 Inclusion/Exclusion Criteria

List Inclusion criteria:

- Liver transplant recipients ≥ 18 years old
- Renal dysfunction (GFR ≤ 60 mL/min) at 30 days post-operatively +/-7 days
- Rabbit anti-thymocyte globulin (rATG) induction (at least 1 dose)
- Indication for transplant: ethanol, hepatitis C, or nonalcoholic steatohepatitis

List Exclusion criteria:

- Increased risk of rejection: autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, positive crossmatch, retransplantation
- Incompletely healed incision or other wound healing issues at time of randomization
- Multiple or previous organ transplantation
- Severe, uncontrolled hypercholesterolemia (> 9mmol/L) or hypertriglyceridemia (>8.5 mmol/L) in the 6 mo prior to transplantation
- Insurance company unwilling to pay for the cost of the everolimus
- Pregnant women
- Unable to provide informed consent

5.0 Enrollment/Randomization

Following transplant, prior to the one month post-transplant visit, all eligible subjects will be approached either in the transplant unit in the hospital or at the transplant clinic in the hospital for study participation. A random permuted block design will be used to generate random assignments to the groups. This method will ensure balance across the groups over the course of the study period. The biostatistician at the Center for Outcomes Research in Surgery will prepare the master randomization assignment list. Clinical research coordinator will then administer randomization. Since this is an open label study, care providers will not be blinded to the randomization. We do not believe this will interfere with the results of the study or patient care.

Following enrollment, subjects will be randomized at one month post transplant to reduced dose Tacrolimus plus mycophenolic acid (control group) or to Everolimus plus mycophenolic acid (study group) maintenance immunosuppression.

A previous Phase III study (Baboolal, 2003) showed that the mean creatinine clearance rate – a measure to assess the kidney function post-transplant – in the treatment group (those receiving Sirolimus and a CNI (Cyclosporine or Tacrolimus) with planned withdrawal of the CNI) was 65mL/min (SD=14) and in the control group (those who continued to receiving Sirolimus and a CNI) it was 57mL/min (SD=13). Therefore, using the two independent-groups and two-tail t-tests, we will need to recruit 46 patients in each group. With an attrition rate of 11% (Baboolal, 2003), we approximately need 104 participants in this study (52 subjects in each arm) to achieve a statistical power of 80% at 0.05 level of significance. Power analysis and sample size calculation were done using G*Power 3.1.9.2.

6.0 Study Procedures

After liver transplant, all patients will receive the standard induction regimen and Tacrolimus monotherapy.

INDUCTION:
Rabbit anti-thymocyte globulin (rATG) 1.5 mg/kg of actual body weight rounded to nearest 25 mg and capped at 150 mg for up to three doses given IV on post-operative day (POD) 1, 3, and 5. Some patients may receive only one dose if they are considered too frail to need all three doses.

30 minutes prior to infusion, pre-medicate with the following:

- Daily steroid dose
- Acetaminophen (Tylenol®) 650 mg PO or per NG x 1 dose
- Diphenhydramine (Benadryl®) 25 mg IV push x 1 dose

Steroids:
Methylprednisolone (Solu-Medrol®) 250 mg IV push x 1 dose on POD 1 (given 30 minutes prior to rATG) and 125 mg IV push x 1 dose on POD 3.

Maintenance:
Low dose Tacrolimus (FK / Prograf®) (titrated to a goal trough of 5 ± 1 ng/mL) plus mycophenolic acid (Cellcept or Myfortic).

RANDOMIZATION:
On POD 30, patients meeting study criteria will be randomized to either the study arm or control arm. Patients randomized to the study arm will be converted to Everolimus (target trough levels 4-8 ng/mL) plus mycophenolic acid (Cellcept or Myfortic) therapy. mycophenolic acid dose as deemed appropriate per standard of care. Everolimus will be initiated at 1mg BID. Tacrolimus will be discontinued once Everolimus level is within goal

range, or at the discretion of the provider. The control arm will be maintained on the low dose Tacrolimus plus mycophenolic acid therapy.

At 3 months, patients with GFR ≤60 will proceed to reduced dose Everolimus (target trough levels 3-6 ng/mL) plus mycophenolic acid therapy. Patients with GFR >60 will proceed to Everolimus monotherapy (target trough levels 4-8 ng/mL).

If deemed clinically appropriate, mycophenolic acid may be held for up to 2 weeks.

Complete blood counts, liver function panels, and drug levels will be monitored as done Standard of Care [SOC]: initially twice per week for first month, once per week for next two months, once every other week for next three weeks, and then once monthly. Ultrasound, ERCP, biopsy as needed by clinical situation as SOC.

7.0 Data to be Collected

Target Population: Liver-only transplant patients

Baseline Information: Demographics (age, race, sex, BMI), pre-treatment medical history, pre-treatment concomitant medication.

Intervention: The treatment period starts at 30 days post transplant. Subjects will be followed for up to a total of 36 months post transplant.

During the treatment period relevant concomitant medications, adverse events, complete blood counts, liver function panels, and immunosuppression drug levels will be collected.

Follow-up: Data will be collected at 6 month, 12 month, 24 month, and 36 month timepoints. GFR will be measured at these time points. Any biopsy confirmed rejection that occurs on or before the 36 month timepoint will be recorded. See Table 1 for timeline for data collection.

Occurrence or change in NODAT [New Onset Diabetes mellitus After Transplant], neurocognitive function, hypertension, malignancy, and tolerance will be recorded throughout the length of the study. Results of any ultrasound, ECRP, or biopsy performed for clinical reasons will be recorded throughout the study.

Study follow up will be conducted per standard of care. Patients will not undergo any additional visits for study purposes during treatment or follow up.

Table 1

	days					Months										
	0	1	7	14	21	30[1]	2[1]	3[1]	4[1]	5[1]	6[1]	9[1]	12[2]	18[2]	24[2]	36[2]
Sr Creatinine	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Creatinine Clearance	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MDRD GFR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Sr Glucose	x					x	x	x	x	x	x	x	x	x	x	x
Urine Protein	x					x	x	x	x	x	x	x	x	x	x	x
Lipid profile	x					x	x	x	x	x	x	x	x	x	x	x
BP	x					x	x	x	x	x	x	x	x	x	x	x
LFT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Everolimus level							x	x	x	x	x	x	x	x	x	x
Tacrolimus level		x	x	x	x	x	#	#	#	#	#	#	#	#	#	#

control patients

1. +/- 7 days
2. +/- 1 month

8.0 **Drug Information**

Please see the "CellCept_PM_E," "Myfortic," "Prograf-Tacrolimus," and the "Zortress Package Insert" attachments.

9.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Definitions of Adverse Events

Adverse Event (AE)

An **adverse event** is defined as untoward medical occurrence associated with the use of a drug or procedure in humans, whether or not considered drug or procedure related. An adverse event can be **ANY** unfavorable and unintended sign, symptom, or disease temporarily associated with the use of a medicinal (investigational) product or procedure, whether or not considered related to the medicinal (investigational) product or procedure (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite'). Examples of Adverse Events that will be recorded in this study are:

- Concomitant illness
- Physical injury
- Events possibly related to medication

- Significant worsening (change in nature, severity, or frequency) of the disease under study or other pre-existing conditions
- Drug interactions
- A laboratory or diagnostic test abnormality occurring shortly after the start of the study that results in the withdrawal of the patient from the study, requires medical treatment or further diagnostic work-up, or is considered by the study investigator to be clinically significant

Adverse events will be graded according to the NCI Common Toxicity Criteria, Version 4.0.

Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence resulting in one or more of the following:

- Results in death or ANY death occurring within 28 days of last dose of study drug (even if it is not felt to be drug related)
- Is life-threatening (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
  NOTE: Hospitalizations that are not considered SAEs are:
  - Hospitalization for elective treatment of a pre-existing condition unrelated to study participation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

Unexpected Adverse Event

An adverse event not mentioned in the package insert or the specificity or severity of which is not consistent with the package insert.

Determining Attribution to the Investigational Agent(s)

Attribution: An assessment of the relationship between the AE and the medical intervention. CTCAE does not define an AE as necessarily *"caused by a therapeutic intervention"*. After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE is clearly **NO**T related
	Unlikely	The AE is **doubtfully related**
Related to investigational agent/intervention	Possible	The AE **may be related**
	Probable	The AE **is likely related**
	Definite	The AE **is clearly related**

Adverse Event (AE) Reporting

Adverse events (AEs) will be reported to the principal investigator immediately and will be recorded from the time of randomization (approximately 30 days after surgery), regardless of whether or not the event(s) are considered related to trial procedures or medications. All AEs considered related to trial medication will be followed until resolution, return to baseline, or deemed clinically insignificant, even if this occurs post-trial.

Reporting to the IRB:

1. Unanticipated problems involving risks to subjects or others will be reported **promptly** to the IRB if they:
    1. were unexpected;
    2. were related or possibly related to study participation; AND
    3. suggests that the research places subject(s) or others at greater risk of harm than was previously known.

If the serious adverse event does not meet all three (3) criteria listed above, the event does not have to be promptly reported to the Indiana University IRB.

2. **Prompt** reporting of unanticipated problems to the IRB is defined as within 5 business days of the study team becoming aware of the event.

10.0 Study Withdrawal/Discontinuation

Subjects may withdraw from the trial at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator for any reason. If the patient withdraws from the trial and also withdraws consent for disclosure of future information, no further evaluations should be performed and no additional data should be collected. The investigator may retain and continue to use any data collected before such withdrawal of consent.

11.0 Statistical Considerations

Univariate analysis will be done to describe the distribution of the sample in terms of demographics (age, race, sex, BMI), pre-treatment medical history, pre-treatment concomitant medication. The distribution of the sample will be reported in terms of mean (±SD), median (Inter-quartile range), or percentage depending upon the type of variable (continuous or categorical). Bivariate analysis comparing the sociodemographic variables of the treatment groups will be conducted using chi squared, student T tests, Mann U Whitney, and ANOVA followed by Tukey's pairwise comparison of the means as appropriate.

Intent-to-treat analysis of the data from the baseline and follow-up visits will be analyzed in order to compare the renal functions after liver transplant between the study arm (those receiving Everolimus + mycophenolic acid) and the control arm (those maintained on the low dose Tacrolimus monotherapy + mycophenolic acid therapy. Repeated measures analysis will be conducted to analyze measures of renal function after liver transplant across the treatment groups using multivariable generalized linear model (GLM) over time accounting for the correlation of the measurements obtained for each patient over time. For the secondary end-points, bivariate analysis will be done to compare the outcomes between the treatment and the control groups. Pearson's chi-square, student T tests, and Mann U Whitney tests as appropriate. Adjustment for the correlated variance due to repeated measures will be made throughout the analysis. All analysis will be conducted at 0.05 level of significance using Stata/SE version 14.2 (StataCorp., 2015).

12.0 Data Management

Primary Data will be collected directly from the Electronic Medical Record databases CERNER, OTTR, and UNOS. Records requests from third party hospitals will be collected as needed primarily for, but not limited to, Adverse Event reporting and medical history. Adverse Events will be recorded on paper CRFs. Each Adverse Event will be reviewed and attributed by the PI. The Adverse Event CRFs will be signed by the PI. Any lab values, drug levels, or other data not recorded in the Electronic Medical Record will be recorded by study personnel on paper CRFs and signed.

Signed consents and HIPAA authorizations will be stored in study binders. Paper copies of record requests, paper CRFs, and any Notes To File will also be stored in these study

binders. Source material from the Electronic Medical Record will be printed only if needed for data quality control checks and stored in the study binders. Study binders will be stored in a HIPAA compliant, locked room.

Data will be stored electronically in REDCap. The REDCap data file will be backed up manually at least every month on a separate, HIPAA-compliant IU Box Health folder. The database will be built and tested by a biostatistician prior to the production mode.

Data collected in the electronic database will undergo random audits to ensure accuracy. A data safety officer (Dr. Asif Sharfuddin from Department of Medicine, Division of Nephrology will kindly serve as the data safety officer and will perform quality control checks on the entered data.

13.0 Follow-up and Record Retention

Subjects will be followed for up to a total of 36 months following transplant. All records produced or collected in connection with this research project, including primary (e.g., laboratory, medical), financial, statistical, supporting, administrative, and regulatory documentation, shall be retained for a minimum period of seven (7) years.

References:

1. P De Simone, F Nevens, L De Carlis, *et al* (2012) "Everolimus With Reduced Tacrolimus Improves Renal Function in De Novo Liver Transplant Recipients: A Randomized Controlled Trial" *Am J Transplant.* 2012 Nov; 12(11): 3008–3020. doi: 10.1111/j.1600-6143.2012.04212.x
2. Saliba F, De Simone P, Nevens F *et al* (2013) Renal function at two years in liver transplant patients receiving everolimus: results of a randomized, multicenter study. Am J Transplant. 2013 Jul;13(7):1734-45.
3. Fischer L, Saliba F, Kaiser GM et al (2015) Three-year Outcomes in De Novo Liver transplant Patients Receiving Everolimus With Reduced Tacrolimus: Follow-Up Results From a Randomized, Multicenter Study. Transplantation. 2015 Jul;99(7):1455-62.

4. Li L1, Wozniak LJ, Rodder S, Heish S, et al. (2012) A common peripheral blood gene set for diagnosis of operational tolerance in pediatric and adult liver transplantation. Am J Transplant. 2012; 12(5):1218-28