Title: A Randomized Prospective Trial of Conversion to Everolimus Therapy from Calcineurin Inhibitor Based Maintenance Immunosuppression in association with Mycophenolic Acid in Liver Transplantation: Examination of Impact on Long Term Renal Function (December 28, 2018)

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1.0 Introduction

Post liver transplant maintenance immunosuppression is based on calcineurin inhibitors, which have a nephrotoxic effect. Calcineurin inhibitors cause microvascular and glomerular damage and are associated with arteriolar hyalinosis and striped interstitial fibrosis of the kidneys. Early calcineurin inhibitor related changes may be reversible but nephrotoxicity with chronic administration of these immunosuppressant agents appears to be irreversible and can sometimes lead to renal transplantation. Significant renal impairment has been observed in 27% of liver transplant recipients at 5 years postoperatively, with up to 10% reaching end stage renal disease. Such late chronic renal failure has been associated with a significant risk of premature death and can be predicted by creatinine levels in the first year post liver transplant. With the implementation of the MELD (Model for End Stage Liver Disease) liver allocation system, more patients are reaching liver transplantation with baseline renal dysfunction as creatinine is heavily weighted within the MELD score.

Everolimus is a macrolide immunosuppressant of the proliferation signal inhibitor class (PSI, mTOR inhibitor). It is an orally active 40-O-(2-hydroxyethyl) derivative of sirolimus, which blocks growth factor-dependent cellular proliferation through a calcium independent signal. The immunosuppressive effect of everolimus is exerted by blocking interleukin 2 and interleukin 15 driven proliferation of hematopoietic (T & B cells) and non hematopoietic (vascular smooth muscle cells) cells by inhibiting the activation of p70S6 kinase. This arrests the cell cycle at the G1 phase thus preventing the progression into the S phase. Both everolimus exerts its inhibitory effect by forming a complex with the immunophilin FK506 binding protein 12. The 50% inhibitory concentration for everolimus inhibition of FK506 binding to FK506 binding protein 12 is 1.8 to 2.6 nmol/L which is approximately 3x higher than that of sirolimus (0.4-0.9 nmol/L).

Interest in mTOR inhibitors, such as everolimus, lies in their potential renal sparing effect in the setting of calcineurin inhibitor maintenance therapy in heart, renal and liver transplantation. Everolimus as the newer of the mTOR inhibitor agents has the advantage of a shorter half life (40 hrs), allowing a once daily oral administration. Data showing improvement of renal function when an mTOR inhibitor completely replaces a calcineurin inhibitor in renal transplantation makes such a strategy particularly attractive as a renal sparing immunosuppressive regimen for liver transplant recipients.

A high proportion of liver transplant candidates on the Penn State Hershey Medical Center waiting list are in renal failure at the time of liver transplant. From September 2005 until January 2011, a total of 111 orthotopic liver transplants were performed. Forty three (39%) of these recipients showed evidence of renal failure in the pre- transplant period with a creatinine level > 2mg/dL. Fifteen patients required intra-operative continuous venovenous hemodiafiltration (CVVHD), 8 required CVVHD pre-transplant and five underwent dialysis in the post transplant period. The predominant cause of renal failure in these patients was hepatorenal syndrome with a prolonged course in some patients due to the long waiting time and organ donor shortage. The postoperative immunosuppressive protocol implemented at Penn State consists of a combination of a calcineurin inhibitor (tacrolimus or cyclosporine), mycophenolic acid and steroids (tapered over the first 3 months).

Given the increasing proportion of patients having renal failure at the time of transplant, associated with the nephrotoxic effect of calcineurin inhibitor based immunosuppression associated with its long term negative survival impact, this study proposes to examine the renal sparing impact of conversion to everolimus from a calcineurin inhibitor based immunosuppressive protocol between 90 days -120 days post liver transplant. 90 days-120 days post transplant was chosen to allow for the switch to everolimus to occur at a period of stable post transplant liver function when both technical and rejection risks are lower. The 90 days-120 days was also chosen because of data indicating that worsening renal function at 4 weeks, 3 months and 1 year post transplant is an independent risk factor for the development of chronic renal failure and end stage renal disease after orthotopic liver transplantation.

2.0 Objectives

Primary:

Examine the renal sparing impact of implementing a strategy of conversion to everolimus from a calcineurin inhibitor based immunosuppressive protocol between 90 days-120 days post liver transplant.

Secondary:

- Evaluate patient and liver graft survival at 6, 12, and 24 months.
- Evaluate incidence and severity of biopsy proven rejection at 6, 12, and 24 months.
- Evaluate the cost-effectiveness of the strategy of conversion to everolimus from calcineurin inhibitor between 90 days-120 days post liver transplant.
- Perform recipient and donor genotyping to examine their impact on optimizing the dosage of everolimus.

3.0 Study Design and Methods

- This is a prospective randomized open label pilot study to assess superiority of immunosuppressive regimen. Patients will be randomized to one of two arms between 90 days-120 days post transplant:
- **Arm A:** Conversion to Everolimus immunosuppression combined with mycophenolic acid (Myfortic: MPA), and complete discontinuation of Calcineurin inhibitor will occur after the target level of Everolimus is reached. .
- **Arm B:** Continuation with standard immunosuppressive therapy consisting of Calcineurin inhibitor associated with mycophenolic acid (Myfortic: MPA).
- Steroids are routinely discontinued at 3 months post transplant as part of the Penn State standard immunosuppressive protocol (Exceptions to steroid discontinuation: Primary diagnosis of autoimmune cirrhosis, primary biliary cirrhosis and primary sclerosing cholangitis).
- Recipients of a deceased donor liver transplant who are 18-70 years of age will be asked between 90 days -120 days post transplant whether they wish to participate in this study.
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4.0 Inclusion and Exclusion Criteria

Study Population: This will be a pilot study which will include 12 adult deceased donor liver transplant recipients in each study arm.

Inclusion

- Ability and willingness to provide written informed consent and adhere to study regimen.

- Recipients who are 18-70 years of age of a primary liver transplant from a deceased donor.
- Allograft is functioning at an acceptable level by the time of randomization as defined by the AST, ALT, Total Bilirubin levels ≤3 times ULN, and AlkP and GGT levels ≤ 5 times ULN. Elevated GGT alone, in combination with AST, ALT, total bilirubin and AlkP within the defined range does not exclude patients from randomization.
- Recipients who have been initiated on an immunosuppressive regimen that contains corticosteroids and tacrolimus or cyclosporine by 7 days post-transplantation.
- Confirmed recipient HCV status at Screening (either by serology or by PCR). Laboratory results obtained within 12 months of screening are acceptable.
- Abbreviated MDRD eGFR ≥ 30 mL/min/1.73m2. Local and central serum creatinine results obtained within 5 days prior to randomization are acceptable, however no sooner than Day 25 post-transplantation.
- Verification of at least one tacrolimus trough level of ≥ 8 ng/mL in the week prior to randomization. Investigators should make adjustments in tacrolimus dosing to continue to target trough levels above 8 ng/mL prior to randomization.
- Patients who are able to take oral medication at time of randomization.

Exclusion

- Patients who are recipients of multiple solid organ or islet cell tissue transplants, or have previously received an organ or tissue transplant. Patients who have a combined liver kidney transplant.
- Recipients of a liver from a living donor, or of a split liver.
- History of malignancy of any organ system within the past 5 years whether or not there is evidence of local recurrence or metastases, other than non-metastatic basal or squamous cell carcinoma of the skin or HCC (see next criteria).
- Hepatocellular carcinoma that does not fulfill Milan criteria (1 nodule ≤ 5 cm, 2-3 nodules all < 3 cm, seen at the time of transplantation as per explant histology of the recipient liver.
- Any use of antibody induction therapy.
- Patients with a known hypersensitivity to the drugs used on study or their class, or to any of the excipients.
- Patients who are recipients of ABO incompatible transplant grafts.
- Recipients of organs from donors who test positive for Hepatitis B surface antigen or HIV are excluded.
- Patients who have any surgical or medical condition, which in the opinion of the investigator, might significantly alter the absorption, distribution, metabolism and excretion of study drug.
- Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, including women whose career, lifestyle, or sexual orientation precludes intercourse with a male partner and women whose partners have been sterilized by vasectomy or other means, UNLESS (1) they meet the following definition of post-menopausal: 12 months of natural (spontaneous) amenorrhea or 6 months of spontaneous amenorrhea with serum FSH levels >40 mIU/m, or (2) have past 6 weeks from surgical bilateral oophorectomy with or without hysterectomy or (3) are using one or more of the following acceptable methods of contraception: surgical sterilization (e.g., bilateral tubal ligation, vasectomy), hormonal contraception (implantable, patch, oral), copper coated IUD and double-barrier methods (any double combination of male or female condom with spermicidal gel, diaphragm, sponge, cervical cap). Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Reliable contraception should be maintained throughout and for 3 months after study drug discontinuation.

- Patients with any history of coagulopathy or medical condition requiring long-term anticoagulation which would preclude liver biopsy after transplantation. (Low dose aspirin treatment or interruption of chronic anticoagulant is allowed).

Enrollment Exclusion - Randomization

- Patients who have severe hypercholesterolemia (>350 mg/dL; >9 mmol/L) or hypertriglyceridemia (>500 mg/dL; >8.5 mmol/L) within 6 months of transplantation. Patients with controlled hyperlipidemia are acceptable at the time of randomization.
- Patients with platelet count < 50,000/mm3 at the time of randomization.
- Patients with an absolute neutrophil count of < 1,000/mm³ or white blood cell count of <2,000/mm³ at the time of randomization.
- Patients who have tested positive for HIV. Negative laboratory results obtained within 6 months prior to transplant are acceptable.
- Patients with clinically significant systemic infection requiring active use of IV antibiotics at the time of randomization. Patients who are in a critical care setting at the time of randomization requiring life support measures such as mechanical ventilation, dialysis, requirement of vasopressor agents.
- Patients who require renal replacement therapy for clearance within 7 days prior to randomization.
- The presence of thrombosis via Doppler ultrasound of the major hepatic arteries, major hepatic veins, portal vein and inferior vena cava. Results obtained within 5 days prior to randomization are acceptable, however no sooner than Day 25 post-transplantation.
- An episode of acute rejection that required antibody therapy or more than one steroid sensitive episode of acute rejection during the run-in period. This includes patients who have not completed steroid treatment for acute rejection within 7 days prior to randomization.
- Patients with a confirmed spot urine protein-to-creatinine ratio that indicates greater than 0.7 mg protein /mg creatinine. Laboratory results obtained after transplant surgery and within 4 months of randomization are acceptable.
- Use of immunosuppressive agents or treatments after baseline that are not utilized in the protocol.

5.0 Recruitment and Consent Process

The primary investigator, Dr Zakiyah Kadry, will obtain consent and discuss in detail the process of randomization and the risks and benefits of the study both at time of admission for transplant and between 90 days-120 days post-operatively. Patients will be provided with a copy of the consent form at time of transplant to allow them to consider participation in the study between 90 day-120 day time point. Patients will also be seen regularly on an outpatient basis for routine follow up in the first 3 months of transplant, at which point any additional questions they may have about the study can be addressed. Between 90 days-120 days post transplant, patients will be seen on an outpatient basis and will be asked if they are interested in participation, during which time the study randomization process and consent process will again be undertaken in detail.

6.0 Study Procedures

Conversion from CNI to Everolimus in Arm A:

Randomization will only occur between 90days and 120 days post transplant. Patients randomized between 90 days and 120 days for conversion to Everolimus, in association with Mycophenolic acid, will start Everolimus at a dose of 0.75mg twice daily, with target levels at 3 to 8 ng/mL. Blood levels of Everolimus will be measured by the FDA approved assay by ThermoFisher. The calcineurin inhibitor (tacrolimus or cyclosporine) will be withdrawn after the target levels of Everolimus are reached.Concurrent immunosuppression with MPA will be maintained in both arms with dosage dependant on the degree of bone marrow suppression and leucopenia.

The calcineurin inhibitor (tacrolimus or cyclosporine) will be withdrawn after the target level of Everolimus is reached.

For Tacrolimus: After everolimus blood trough levels have been confirmed to be in the target range (3-8 ng/mL), tacrolimus tapering will begin, targeting a tacrolimus whole blood trough level of 3-5 ng/mL by three weeks after randomization. Once that point is reached tacrolimus elimination will begin while maintaining everolimus whole blood trough levels within the target range of 6-8 ng/mL.

For Cyclosporine: After everolimus blood trough levels have been confirmed to be in the target range (3-8 ng/mL), cyclosporine tapering will begin, targeting a cyclosporine whole blood trough level of 50 to 100 ng/mL by three weeks after randomization. Once that point is reached cyclosporine elimination will begin while maintaining everolimus whole blood trough levels within the target range of 6-8 ng/mL.

Prior to CNI elimination, the inclusion criteria for liver function tests will be re-applied. If a patient does not exhibit a functioning allograft at an acceptable level by as defined by the AST, ALT, Total Bilirubin levels being ≤3 times the upper limits of the normal values (ULN) and Alkaline Phosphatase and Gamma Glutamyl Transferase levels ≤5 times ULN, then the patient will be monitored until these criteria are met, after which tacrolimus/cyclosporine elimination can begin.

Everolimus and CNI trough concentrations will be measured twice a week for the first 6 months after transplant. After 6 months, trough concentrations may be measured on a weekly basis until end of month 12 post transplant, after which levels maybe measured on a q2weekly basis. Patients seen in the post liver transplant clinic may have an additional measurement performed that day in conjunction with their routine laboratory work.

Target levels for Arm B:

The most common CNI used at our institution is prograf (Tacrolimus) & at 3 months the level is maintained at 10-12 ng/mL unless the patient has hepatitis C where the levels are kept at a slightly lower level of 8-10 ng/mL. Concurrent immunosuppression with MPA will be maintained in arm B with dosage dependant on the degree of bone marrow suppression and leucopenia. If cyclosporine is utilized, the level at 3 months is maintained at the 200 to 250 ng/mL range unless the patient has hepatitis C where the levels are kept at a slightly lower level of 150 to 200 ng/mL.

Study duration:

Start Date: 1 October 2012

End Date: 1 October 2019

2 year subject enrollment..

Evaluation schedule:

Visit number	1 Screening*	2 Baseline*	3	4	5
Time of Visit	90days-120 days post transplant	90days-120 days post transplant	Month 6 +/- 2 weeks***	Month 12+/- 2 weeks ***	Month 24+/-2 weeks ***
Inclusion/Exclusion criteria	X	x			
Informed consent	X				
Physical examination(medical history and vital signs	X	x	x	x	x
Clinical assessment	X	x	x	x	x
Quality of Life Questionnaires (FACT-Hep; EQ-5D)	X	X	X	X	X
Dispense study medication		x			
Return study medication					x
Laboratory test (blood & urine collection)	X	x	x	x	x
Adverse events		x	x	x	x
Blood test-HIV, hepatitis viruses, CMV and genotyping	X				
Pregnancy Test	X				
Concomitant Medications (Standard of Care) **					
Valcyte	X	X			
Bactrim(PCP prophylaxis)	X	X	X	X	
Mycophenolic acid (Myfortic)	X	X	X	X	X

* If the patient meets all inclusion criteria at the Screening Visit, the Screening and Baseline visits may occur on the same day and will occur between 90 days and 120 days post transplant.

**All patients receive CMV prophylaxis with Valcyte in the 1st 4 postoperative months as part of standard care. Bactrim prophylaxis against Pneumocystis carinii is administered for the first year post transplant as part of standard care. Steroids are discontinued at 3 months post-transplant while Myfortic is continued as part of the maintenance immunosuppressive protocol.

***Visit window for follow up visits at months 6, 12 and 24 is plus or minus 2 weeks

Tests at above evaluation schedule:

a. Renal function as assessed by the iothalamate clearance test starting at Baseline visit.
b. Renal Function as assessed by serum creatinine, by Cockcroft-Gault formula mL/min, and 24 hour urine collection for creatinine clearance and urinary protein level starting at Baseline visit.
c. Liver Recipient blood sample for genotype testing.
d. Testosterone levels

The genetic tests will involve TaqMan real-time PCR of extracted DNA, and subsequent identification of alleles associated with investigated polymorphic SNP by means of allele-specific 5' nuclease assay, using the pre-developed assay and Custom TaqMan® SNP Genotyping reagents for allelic discrimination by TaqMan real-time PCR on AbiPrism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The above will involve following genes and polymorphisms: CYP3A5

gene: 6986 A>G (alleles *1 and *3) and ABCB1 (MDR1) exon26 (3435C>T) and exon21 (2677G>T/A).

7.0 Risks and Discomforts

The following adverse reactions have been described with everolimus: Leukopenia, the development of increased cholesterol and hyperlipidemia (high blood fats) levels, thrombocytopenia, anemia and a predisposition for viral, bacterial and fungal infections due to the immunosuppressant effect of everolimus. Hemolytic uremic syndrome, surgical wound complications, venous thromboembolism, acne and hypertriglyceridemia have also been described. Additional common side effects observed in transplant patients taking everolimus include hypertension, gastrointestinal disorders and edema. In two adult kidney transplant studies, less than 5% of the patients exhibited the combination of low testosterone and sexual dysfunction as an adverse event. However, the clinical implications of this are not clear.

Side effects of calcineurin inhibitors such as tacrolimus and cyclosporine are well described and include nephrotoxicity, neurotoxicity, hyperkalemia, and hypomagnesemia. Cyclosporine also causes hypertension, hepatotoxicity, hirsutism, hypertension, hyperlipidemia, gingival hyperplasia and sinus congestion. Unlike cyclosporine, Tacrolimus causes alopecia, may have a diabetogenic effect and causes less disturbances in lipid metabolism. Allergic reactions can occur with tacrolimus depending on the formulation, especially if castor oil is used.

Side effects of Iothalamate clearance test:
Rare allergic reactions to iothalamate in subjects known to be allergic to iodine. Can range from urticaria to anaphylactic shock. Other adverse effects of iothalamate include arrythmias, bradycardia, generalized vasodilation, extravasation of iothalamate during injection, seizures, and weakness of extremities. Abnormal sensation or muscle spasm in the injection site has been reported but in patients being infused with five to thirteen times the amount planned in this study and at a faster infusion rate.

Mycophenolic acid has bone marrow suppression as the main side effect and rarely may have neurotoxicity associated with it. It is also an enteric coated formulation and hence should have less of the GI side effects such as nausea, vomiting and diarrhea, than mycophenolic mofetil which is a similar drug.

Given that arm A will consist of the patients on everolimus and mycophenolic acid, with complete withdrawal of calcineurin inhibitor, there will be a risk of bone marrow suppression and leucopenia, which will be dosage dependant.

Both calcineurin inhibitors and mycophenolic acid increase the patient risk for cancer, especially post transplant lymphoproliferative disease. Everolimus is thought to have an antiproliferative effect and may reduce the risk for the development of cancer.

All patients will be monitored closely for any of the above side effects. Other than the scheduled study clinic visits and testing, allpatients will be followed as per the Penn State Division of Transplantation follow up guidelines. For the first 24 months post liver transplant all our patients undergo the following routine lab collection procedure, the frequency of which may be adjusted based on patient clinical status and need:

Routine/standard of care laboratory tests from post op month 1 through post op month 24 include: :

- ✓ 1st 5 months postop: 2x/week labs as follows: Sodium, Potassium, Chloride, Bicarbonate level, BUN, Creatinine, Magnesium, Calcium, Phosphorus, CBC, PT, INR, PTT, Liver Function Tests (Total & Direct Bilirubin, AST, ALT, Alkaline Phosphatase & Gamma Glutamyl Transferase), Glucose & Immunosuppressive agent trough levels. Lipid Panel to be performed once a week.

- ✓ Month 6 to 12 inclusive: q weekly labs: Sodium, Potassium, Chloride, Bicarbonate level, BUN, Creatinine, Magnesium, Calcium, Phosphorus, CBC, PT, INR, PTT, Liver Function Tests (Total & Direct Bilirubin, AST, ALT, Alkaline Phosphatase & Gamma Glutamyl Transferase), Glucose, Immunosuppressive agent trough levels and Lipid Panel.

- ✓ After month 12: q2weekly labs: Sodium, Potassium, Chloride, Bicarbonate level, BUN, Creatinine, Magnesium, Calcium, Phosphorus, CBC, PT, INR, PTT, Liver Function Tests (Total & Direct Bilirubin, AST, ALT, Alkaline Phosphatase & Gamma Glutamyl Transferase), Glucose, Immunosuppressive agent trough levels and Lipid Panel.

Any abnormal laboratory values routinely result in a complete evaluation of the patient which may also include radiologic studies and a liver biopsy. Dose adjustments &/or discontinuation of medications may be required.

Years 2 & 3 Post Liver Transplant:

At 24 months post transplant patient's will have their final study visit and will be followed by the Divisions of Tranplant and Hepatology per standard of care. Labwork includes: Sodium, Potassium, Chloride, Bicarbonate level, BUN, Creatinine, Magnesium, Calcium, Phosphorus, CBC, PT, INR, PTT, Liver Function Tests (Total & Direct Bilirubin, AST, ALT, Alkaline Phosphatase & Gamma Glutamyl Transferase), serum Glucose, Immunosuppressive agent trough levels and Lipid Panel.

Clinic Visits:
Year 1:
1x/week x 1 month
Every 2 weeks x 1 month
If stable: clinic visit x 1 at 1, 3, 6 & 9 months

Any persistent elevation in liver function tests (on consecutive testing within 1 week) or clinical issues: patient is brought to clinic regardless of scheduled clinic visits.

Years 2 & 3 inclusive:

Clinic every 3 months, unless elevation in liver function tests (persistent on 2 consecutive tests over a 1 week period) or if medication change or if clinical symptoms/complaints. If the latter occurs, patients should be seen in clinic during that week.

All patients undergo a full history and physical examination during their clinic visit associated with a complete review of their laboratory results and their current medications.

In this study, patients will be monitored for the development of medication side effects:

Everolimus & Mycophenolic Acid:

For patients who are unable to tolerate the protocol-specified dosing schedule of everolimus, dose adjustments will be made according to the following guidelines:

The everolimus dose will be decreased by at least 0.25 mg b.i.d if a dose reduction is necessary. If a temporary reduction in everolimus level is needed, the everolimus trough blood level will be maintained no lower than 3 ng/mL. Everolimus will be discontinued if a trough level ≥ 3 ng/mL cannot be maintained due to toxicity. Severe and unremitting changes/side effects may also lead to everolimus discontinuation.

If everolimus is interrupted for safety reasons for longer than 21 consecutive days, or for more than 2 episodes of 7 days or longer, discontinuation of study medication will be maintained and the patient will be removed from the study.

During the course of the trial everolimus will be administered on a b.i.d. regimen. Everolimus will not be provided to patients after they have discontinued the study.

If reduction of everolimus is necessary for the patient to remain in target trough range, everolimus dosing will be reduced by 0.25 mg intervals.

In cases of hyperlipidemia, lipid lowering therapy will be optimized before dosage reduction of study medication is considered. Patients will be managed with HMG Co-A reductase inhibitors (e.g., fluvastatin). Lovastatin or simvastatin will not be utilized. The starting dose of HMG Co-A reductase inhibitors will be equivalent to 20 mg pravastatin or 20 mg fluvastatin, and may be increased to target with the aim of maintaining a LDL level of < 130 mg/dL. Patients will be monitored for the development of rhabdomyolysis or other adverse events.

Both everolimus and mycophenolic acid have a bone marrow suppressant effect and predispose patients to neutropenia. Patients who develop neutropenia with an absolute neutrophil count (ANC) <1000 will be initially treated with administration of neupogen (filgrastim) at a dose of 5mcg/kg/day subcutaneously prior to dose reduction of immunosuppressant agents. If there is no response to neupogen therapy after 3 consecutive treatment days, dose reduction of mycophenolic acid will first be undertaken. If the ANC persists at <1000 after reduction of mycophenolic acid, a dose reduction in everolimus should be undertaken as outlined below. .

Guidelines for everolimus dose reduction/interruption:

	Platelet count (/mm3)	Hemoglobin (g/dL)	ANC count (K/UI)	Cholesterol (mmol/L)	Triglycerides (mmol/L)
Dose reduction	≤ 75,000	≤ 8	≤ 1000	≥ 8	≥ 6.5
Interruption	≤ 50,000	≤ 6	≤ 500	≥ 9	≥ 8.5

All patients will be carefully assessed for any of the side effects or adverse reactions related to everolimus through a detailed history and physical examination during clinic visits as listed above and through a thorough and complete examination of their routine and study laboratory values.

A foley catheter placement is required during iothalomate testing to accurately measure urine output collection. Risks of foley catheter placement include: pain, hematuria, injury to urethra, urinary retention following removal of foley, urinary tract infection and bladder spasms.

Patients will be withdrawn from the study in the event of a serious life threatening adverse event.

8.0 Benefits

Everolimus has a profile of anti-lymphoproliferative and anti-angiogenic effects in addition to its immunosuppressant effects and lacks nephrotoxicity. The main benefit of this study relates to renal sparing in patients in arm A, who will be converted from a calcineurin based immunosuppressive protocol to everolimus in combination with mycophenolic acid between 90 days-120 days post operatively. Thus the main benefit which is being evaluated in this study will be improvement and stability in renal function compared to patients on long term calcineurin inhibitors, which in the long run is believed to result in better long term survival outcomes.

9.0 Reporting of Adverse Events and Unanticipated Problems Involving Risks to Participants or Others

All adverse events (AE) will be documented on study specific case report forms and entered into a computer based log. All deaths on study, not related to progression of underlying disease, will be reported to the IRB and study sponsor immediately. All unanticipated AEs related or possibly related to the study will be reported by the PI to the IRB according to HSPO policies and procedures.

10.0 Study Withdrawal/Discontinuation:

This is a small pilot study (12 randomized patients in each of two groups) and in view of the small patient cohort. Stopping rules for this study will therefore be based on patient safety.

Monitoring Process:

Primary monitoring of the study will be performed by the study team, who will review accrual, adverse events, and dose limiting toxicities on a real-time basis. Cumulative adverse events and study toxicities will be reviewed weekly. The study team will make decisions regarding dose escalation, cessation of accrual, and whether stopping rules have been reached. A Data Safety Monitoring Board (DSMB) has been created and its members are listed in the study protocol at the end of Section 13. The DSMB will review the study progress reports provided to the DSMB by the study team and a statistician. A DSMB report will be generated by the DSMB and forwarded to the IRB and the PI. A copy of any interim DSMB reports will be included with continuing review reports submitted by the PI for IRB review.

If stopping rules have been reached, this will be communicated to the IRB immediately.

Serious adverse events that would qualify for patient removal from study would include:

- Refractory leucopenia ≤ 500 unresponsive to drug dose reduction/interruption or stimulation by Neupogen (filgrastim).
- Persistant thrombocytopenia at <50,000/mm3, unresponsive to study medication dose reduction/interruption.
- Refractory hyperlipidemia (Cholesterol ≥ 9 mmol/L or Triglycerides ≥ 8.5 mmol/L) unresponsive to treatment (as outlined in section 7 of the protocol) and to study medication dose reduction/interruption.
- Refractory anemia (Hemoglobin ≤ 6 g/dL) not related to surgical bleeding, and unresponsive to blood transfusion and to study medication dose reduction/interruption.
- Development of hemolytic uremic syndrome
- Development of any thrombotic events such as liver graft hepatic arterial or portal/hepatic venous thrombosis, or thrombotic microangiopathy or thrombotic thrombocytopenic purpura, or radiologically documented pulmonary embolism.
- Refractory seizures or neurologic side effects unresponsive to treatment or reduction/interruption of study medication.
- Life threatening infectious complications.

Reasons for discontinuation of the study drug everolimus will include:

· AE(s)
· Abnormal laboratory value(s)
· Abnormal test procedure result(s)
· Unsatisfactory therapeutic effect
· Significant protocol deviation
· Withdrawal of consent
· Lost to follow-up
· Death

· Graft Loss/Re-transplant

Premature Patient withdrawal:

Patients may voluntarily withdraw from the study or be dropped from it at the discretion of the investigator at any time. Patients will be withdrawn from the study if any of the following occur:

- Withdrawal of consent
- Lost-to follow-up
- Graft loss/re-transplant
- Death
- Serious life threatening adverse event.

If such withdrawal occurs, or if the patient fails to return for visits, the primary reason for a patient's premature withdrawal from the study will be recorded.

For patients who are lost to follow-up, "due diligence" will be shown by documenting in the patient record steps taken to contact the patient, e.g. dates of telephone calls, registered letters, etc.

Patients who are prematurely withdrawn from study medication or from the study after randomization will not be replaced. Patients who withdraw or screen fail prior to randomization will be replaced.

Patients who prematurely discontinue everolimus will be followed for any SAE occurring within 30 days following the last dose of everolimus. These SAEs will be reported on the SAE form.

Patients who become pregnant while taking everolimus or are taking a prohibited immunosuppressive medication not allowed by the protocol will be discontinued from the investigational drug and from the study.

If the everolimus trough level ≥ 3 ng/mL cannot be maintained, patients will be discontinued from the study.

11.0 Statistical Analysis of the Study

The primary endpoint of the trial is a renal function as assessed by 24 hour creatinine clearance.

We will compare creatinine clearance between the two arms of the study using a t-test. We expect that due to randomization there will be no differences in baseline characteristics of the two groups, so no multivariate analyses are planned. However, if we find significant difference between the two groups in such variables as demographics or disease characteristics, we will control for these variables in a linear regression analysis. In addition, we will also explore the use of nonparametric tests (i.e. Mann-Whitney U test for renal function) because of the relatively small sample size.

Secondary endpoints will also be tested using univariate statistical tests. The presence of acute rejection will be compared between study arms using chi-square tests. We will also fit nonparametric survival models to the time to acute rejection to determine whether rejection occurs earlier or later between study arms. Patient and graft survival will be modeled using Kaplan-Meier curves, with comparisons made between arms using the log rank test. To study the effect of donor and recipient genotype testing on postoperative renal function we will study the interaction between treatment arm and presence of CYP3A and P-gp SNPs on creatinine clearance. This will be done using a one-way analysis of variance with main effects for treatment and SNPs, and interaction terms between treatment and the SNPs.

Safety endpoints are all binary indicators (discontinuation, change of therapy, hyperlipidemia, etc.). All of these safety endpoints will be compared between treatment arms using chi-square tests.

We will use the clinical trial data to determine whether the everolimus approach is cost-effective relative to the calcineurin inhibitor based regimen. Costs will be estimated from the perspective of the provider and the time horizon will cover the period of the trial. Costs will be obtained from the hospital's cost-accounting database and will include fixed and variable direct medical costs. Our effectiveness measure for the base case analysis will be renal function either as a continuous measure or in categorical increments (e.g. 5 or 10 ml/min). As a secondary measure of effectiveness we may also study life years gained (YLG), but only if substantial differences are observed in the sample. This will be estimated by the area under the survivor functions for each treatment arm.

Cost-effectiveness analysis asks whether one intervention provides value for resources expended compared to another intervention and is appropriate when alternative interventions have differing implications for costs and patient outcomes. The cost-effectiveness of the everolimus strategy will be summarized with an incremental cost-effectiveness ratio (ICER).

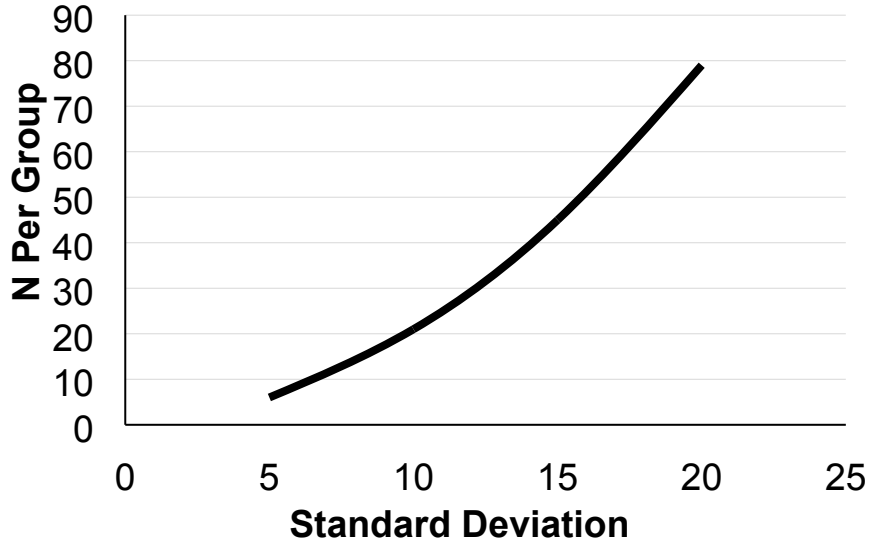
$$ICER = \frac{C_e - C_{ci}}{E_e - E_{ci}} \quad (1)$$

where C_e the cost of the everolimus strategy, C_{ic} is the cost of the calcineurin inhibitor strategy, E_e is the effectiveness of the everolimus strategy, and E_{ci} is the effectiveness of the calcineurin inhibitor strategy. Although we are interested in YLG gained as a measure of effectiveness it is not likely that with 24 patients the difference will be substantial enough to use. Therefore, our primary measure of effectiveness will be units of improvement in renal function. As a secondary measure we will use YLG if large enough differences are observed in our sample. The ICER represents the incremental cost required to achieve one additional unit of benefit if patients are transtioned from cacineurin inhibitors to everolimus. The uncertainty surrounding the ICER estimates will be quantified by bootstrapping our cost and effectiveness data to form 95% ellipses around the cost-effectiveness ratio[25-27]. We will also estimate cost-effectiveness acceptability curves to show how the likelihood of cost-effectiveness varies with the willingness to pay of the decision maker [28].

For all statistical analyses we will define significance as p-values less than 0.05. If multiple comparisons are required (for example, to study the same variable at difference time points) then we will adjust the p-values using a Bonferroni correction.

Sample size and power:

The study was powered to measure significant improvements in renal function. To estimate the required sample size we assumed that the estimated glomerular filtration rate (eGFR) would improve from 34 mL/min/1.73 m^2 to 43 mL/min/1.73 m^2 as reported in a recent study by Fairbanks et al [29]. This is a conservative estimate since the renal sparing effects of everolimus are larger than those of sirolimus. A sample size of 12 in each group will have 80% power to detect a difference in means of -9.0 (the difference between a Group 1 mean of 34.0 and a Group 2 mean of 43.0) assuming that the common standard deviation is 7.5 using a two group t-test with a 0.050 two-sided significance level. Fairbanks did not report standard deviations for eGFR, however, a simulation study suggests that the standard deviation at baseline was 7.5, and the standard deviation following conversion was 16. We therefore, considered the sample size requirement for a range of standard deviations, shown in the Figure below. If the common standard deviation was 16, then 51 patients per group would be required to detect a significant difference of 9.0 with 80% power. This sample size may not be feasible for a pilot study such as this. However, 12 per group is feasible and can be justified by the lower limit of standard deviation reported in Fairbanks.



12.0 Privacy and Confidentiality Considerations

All data forms and study specific information will be kept in locked file cabinets and in a password protected computer database with access limited to the PI, clinical research nurses, and sponsor designees. Any presentation or publication of the data will be done in aggregate fashion without identifiers.

A Data Safety Monitoring Board (DSMB) will review the study progress reports provided to the DSMB by the study team and statistician. A DSMB report will be generated by the DSMB and forwarded to the IRB and the PI. A copy of any interim DSMB reports will be included with continuing review reports submitted by the PI for IRB review.

13.0 Data and Safety Monitoring Plan:

This study is a prospective randomized open label pilot study examining the impact of conversion to everolimus therapy from standard calcineurin inhibitor immunosuppression. Oversight for the conduct of the study will be provided by the PI, Zakiyah Kadry MD, and the clinical research nurse. They will ensure that all eligibility and consent requirements are met prior to a subject's participation on study and that all study procedures and adverse event reporting occur according to the IRB approved protocol. They will follow all study participants, while on study treatment, on a real-time basis for development of adverse events and study end points, utilizing scheduled and as needed physical examinations and laboratory studies. They will make certain that all adverse events are recorded and reported according to the Penn State HSPO (Human Subjects Protection Office) guidelines and that the IRB is notified in a timely manner of any significant safety or data concerns which arise from study review.

Primary monitoring of the study will be performed by the study team, who will review accrual, adverse events, and dose limiting toxicities on a real-time basis. Cumulative adverse events and study toxicities will be reviewed weekly. The study team will make decisions regarding dose escalation, and cessation of accrual.

A Data Safety Monitoring Board (DSMB) will review the study progress reports at six month intervals, with study progress reports provided to the DSMB by the study team and statistician. A DSMB report will be generated by the DSMB and forwarded to the IRB and the PI. A copy of any interim DSMB reports will be included with continuing review reports submitted by the PI for IRB review.

14.0 Compensation

For patients randomized into Group A who are receiving Everolimus medication will be reimbursed for travel expenses in order to return twice per week during the first 3 months after randomization to have the Everolimus blood level checked at Hershey Medical Center's Laboratory. The reimbursement for travel expense is 24 cents per mile based on the 2013 Internal Revenue Service standard mileage reimbursement rate for medical travel and the total repayment will not exceed $300.00. If the patient does not complete the study for any reason during the first 3 months after randomization, the patient will be paid for the medical travel expense up to the time of study discontinuation.

For patients randomized into Group B who are receiving Tacrolimus or Cyclosporin and Mycophenolic acid medications will not receive any payment or compensation for being in this research study because they may continue to have medication blood levels checked at the local laboratory with blood level results faxed to Dr. Kadry's office

15.0 Drugs, Biologics, or Devices

Everolimus (Zortress)
IND# 113882
0.25mg-0078-0417-20
0.5mg-0078-0414-20
0.75mg-0078-0415-20

Investigational Drug Service Pharmacy stores & dispenses investigational item

16.0 Records and Study Monitoring-

Primary monitoring of the study will be performed by the study team, who will review accrual, adverse events, and dose limiting toxicities on a real-time basis. Cumulative adverse events and study toxicities will be reviewed weekly. The study team will make decisions regarding dose escalation, cessation of accrual, and whether stopping rules have been reached. If stopping rules have been reached, this will be communicated to the IRB immediately

17.0 Facilities

Penn State Hershey Medical Center: at the UPC Surgery Clinic facilities & hospital inpatient area if patient is hospitalized.

18.0 References

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