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Proposal Template

Title of study: Safety and efficacy of everolimus transition in minimizing progressive graft dysfunction and interstitial fibrosis in adult kidney transplant recipients

Study purpose and rationale:

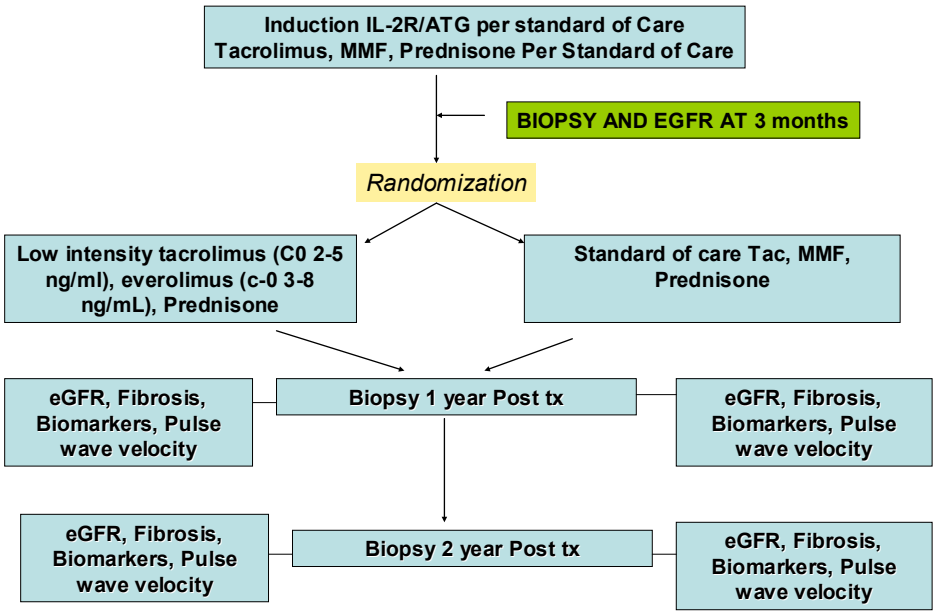
Significant improvements in short term graft survival have been achieved since the introduction of calcineurin inhibitors (CNI) mostly due to a decrease in acute rejection rates in the first year post-transplantation.[1] Despite dramatic improvements in short term survival as a result of decreased acute rejection rates, commensurate improvements in long-term graft survival has not accrued.[2,3] Irreversible and progressive pathologic processes leading to graft fibrosis and eventual graft loss are believed to be the destiny of many grafts and many mechanisms may underlie graft loss.[4]

Unfortunately, current immunosuppressive therapies are not directly aimed at preventing or treating chronic graft injury mostly due to the lack of understanding of the underlying pathophysiological mechanisms. A growing body of evidence demonstrates associations of many immune and non-immune factors that either initiate or participate to some degree in this process of inexorable graft fibrosis.[5-7] The development of fibrosis likely reflects the dynamic interplay mediating the balance between molecules that are pro-fibrotic (TGF-beta) and anti-fibrotic (BMP-7) in their primary action(s).[6] While immune mediated factors like cellular and/or humoral (either subclinical or clinical graft injury) may be initiators of fibrogenesis, non immune mediated factors are also likely to be major pathogenic contributors in this process. Of the non-immune factors, CNI nephrotoxicity is known to be one of the strongest contributors to non-immune graft injury and fibrosis.[8] CNI nephrotoxicity may contribute to attrition of function of the renal allograft by inducing graft fibrosis, loss of functional tissue and eventually, graft loss.[9] The relationship between CNI concentration, dose, and intrinsic propensity of the allograft to CNI mediated injury and the ultimate burden of CNI mediated injury is complex. Naesens et al have demonstrated that vascular and interstitial lesions in allografts are most severe when early post-transplant trough concentrations of tacrolimus were low.[10] The propensity of the allograft to CNI toxicity is not uniform, i.e., peripheral concentrations of drug may not relate to injurious or fibrotic events in the allograft in a dose-linear manner. The risk of acute rejection is higher when therapeutic concentrations of tacrolimus are lower in the early post transplant period. In particular, bioavailability of tacrolimus may be lower in significant subsets of populations, such as African-Americans, who are more likely to carry the expresser phenotype for CYP3A5, which leads to rapid metabolism of substrates with subsequent reduced exposure.[11-13] Recent studies also point to polymorphism in the ABCB1 (P-glycoprotein) gene as an important contributor to the burden of histologic injury, likely as an effect on intra-renal concentrations of tacrolimus.[14-17] To add a further layer of complexity, certain alleles of the apolipoprotein L1 (APO-L1) gene in the donor may confer risk for progressive allograft loss independent of acute rejection, reflecting either intrinsic proclivity to progressive non-immune injury or an inability to fully compensate for or recover from ongoing injury mediated by other mechanisms.[18,19]

Thus far, clinical efforts aimed at minimizing progressive attrition of renal function in the renal allograft have focused on minimizing, avoiding or withdrawing the use of CNIs in renal transplantation. De-novo use of CNI-free regimens that used sirolimus (SRL) and mycophenolate mofetil (MMF) with corticosteroids (CS) met with some success in renal transplantation in single center studies.[20,21] Unfortunately, these efforts were not mirrored to the same degree in the diverse multicenter practice driven outcomes that are reflected in analysis of SRTR data.[22] It should be noted at this juncture that none of the studies mentioned above accounted for the complexity of interactions that likely underlie graft injury and propensity to progressive graft dysfunction reviewed above.
Recent experience with everolimus used with low intensity tacrolimus and corticosteroids may suggest that excellent renal function outcomes accrue without an increased risk of acute rejection (US 92 study).[23] However, the enrollment of higher immunologic risk groups such as African-Americans was lower in the context of this study than is the prevalent norm in many North American centers such as ours.

Studies such as the Rapamune Maintenance Regimen (RMR) study and the Spare the Nephron Study (STN) have shown some promise in affording protection of renal function by switching in stable renal transplant recipients from a CNI-based regimen to a sirolimus, MMF and prednisone regimen at a defined point of 3-6 months post-transplantation.[24-26] The rationale therein being that early acute complications attributable to mTOR inhibition along with the confounding effects of early immunologic events would not obfuscate the gains in renal function expected to accrue from minimization of CNI. There remains however a legitimate concern that any immunosuppression-related drug conversions may be associated with a finite, but relevant, acute rejection risk. This theoretical increased risk could either be due to a preexisting but clinically undetected rejection process at the time of switch or rejection that may be occurring *de novo* after the conversion. In this regard, renal biopsies afford a valuable opportunity to demonstrate absence of acute rejection at the time of minimizing CNIs. The renal biopsy also yields an excellent baseline estimate of the burden of chronic histologic damage. When coupled with an implant biopsy, the rate of change of chronic allograft pathology (interstitial fibrosis and tubular atrophy can also be calculated).[27,28] At the same time, there are currently no robust non-invasive monitoring tools that can be used to determine which particular subject is undergoing either an alloimmune process or a non-immune, drug-induced fibrotic process. There is therefore a need to develop non-invasive clinically useful tools that could eventually permit the clinician to determine the underlying mechanism mediating graft injury and facilitate informed individualization of care.[29]

We hypothesize that transition from tacrolimus based triple therapy with MMF and steroids in stable renal transplant patients to low intensity tacrolimus, everolimus and prednisone will be associated with improvement in GFR and allograft fibrosis. We will test this hypothesis in the context of a randomized, open label, parallel arm study depicted in the schema below. The primary, secondary, exploratory objectives and endpoints that will be examined are detailed further in subsequent sections.



Objectives:

Primary objectives:

1. Measure and compare the change in interstitial fibrosis (morphometric analysis of trichrome stained slides) at one-year post-transplant in patients converted to everolimus, low intensity tacrolimus and prednisone versus the standard of care tacrolimus, mycophenolate and prednisone regimen.

Secondary objectives:

1. Measure and compare the estimated GFR (using the 4-variable modified MDRD equation) in patients converted to everolimus, low intensity tacrolimus and prednisone versus the standard of care tacrolimus, mycophenolate and prednisone regimen, both at one year and two years post-transplant.

2. Compare the patient and graft survival rates at one-year post-transplant in each group.

3. Measure and compare the rates of immunosuppressant discontinuation and modification for each group.

4. Measure and compare the incidence of significant immunosuppressant-related adverse drug reactions for each group.

5. Compare the rates of post-transplant infections, including CMV, BK, and admissions to the hospital for infectious causes in each group.

Endpoints 6 through 10 are exploratory:

6. Measure and compare the rates and quantity of *de novo* donor specific antibody formation at one-year post-transplant in each group; this will address the important clinical question of the risk of humorally mediated alloimmune events with CNI minimization.

7. Determine the impact of gene variants for donor APOL1, donor ABCB1, recipient cytochrome P450 3A5, and donor and recipient angiotensin receptor and angiotensin converting enzyme on the primary objectives. This endpoint addresses pathways that could potentially impact outcome but are not measured by traditional risk factors and explanatory variables such as rejection and immunosuppressant concentrations.

8. Examine changes in gene expression and urinary proteomics using high throughput methods on peripheral blood, renal biopsy tissue and urine. The rationale underlying this endpoint is that these markers could yield potential biomarkers that may be used as a companion diagnostic aid in selecting patients at particularly high risk for renal progression or CNI nephrotoxicity.

9. Examine impact of the everolimus based regimen on cardiovascular health as measured by pulse wave velocity and augmentation index as derived from applanation tonometry. It is expected that the superior GFR, improved blood pressure and decreased sympathotonia expected with everolimus based tacrolimus minimization will improve indices of vascular stiffness and in turn, overall cardiovascular health.

10. An exploratory objective will examine the tissue expression (immunohistochemistry) TGF beta (marker of Epithelial to Mesenchymal transformation (EMT), a precursor of fibrosis and Bone Morphogenetic Protein-7 (BMP-7; Marker of anti-fibrotic events).

Population: Sixty adult renal transplant recipients that give informed consent and meet inclusion and exclusion criteria will be enrolled and randomized in this trial, 30 patients in each group. Patients will be screened, approached, and consented between 10 weeks and 16 weeks post-transplant and randomized at approximately 12 weeks post-transplant to either continue on standard immunosuppressant therapy (control arm) or transition from mycophenolate to everolimus, with a significant reduction in tacrolimus dose and goal trough concentrations. Patients that are screened and consented, but are not randomized to a group will be replaced, such that there will be 30 patients in each group. Once patients are randomized and placed in a study group, they will be followed per study protocol and the analysis will occur in an intent-to-treat methodology.

Inclusion criteria:

1. At least 18 years of age and able to give informed consent.
2. Received a first or repeat cadaveric (including ECD) or living donor renal transplant.
3. Patient has stable graft function, defined as no change of greater than 40% of baseline serum creatinine and no acute rejection during the past month.
4. Currently receiving tacrolimus, mycophenolate mofetil and prednisone as their immunosuppression regimen

Exclusion criteria:

1. Biopsy proven acute rejection episode that occurred within the past month.
2. Malignancy within the past 3 years, except for non-melanoma skin cancer.
3. Currently enrolled in an investigational drug trial.
4. Woman of child bearing potential not utilizing an effective form of birth control.
5. Patients with uncontrolled dyslipidemia, defined at serum fasting LDL >200 mg/dL or serum fasting triglycerides >500 mg/dL.
6. Patients with a spot urine protein to creatinine ratio of > 800 mg of protein per gram of creatinine.
7. WBC < 2,000 cells/mm^3
8. Platelets < 75,000 cells/mm^3
9. Patients who have received an organ transplant other than a kidney.
10. Patients with a history of biopsy proven FSGS, MPGN, or PGN.

Number of centers & patients: Single-center study, enrolling 60 consecutive kidney transplant recipients that meet criteria, give informed consent, and are randomized at 12-weeks (10-16 week timeframe) to either treatment group or control group..

Investigational and reference therapy:

Patients will be randomized to the standard immunosuppression arm (control arm) or the investigational immunosuppression arm (investigational arm). Randomization will occur once written informed consent has been obtained, patient meets inclusion and exclusion criteria and is three months post-transplant.

- **Arm 1 (standard of therapy):** tacrolimus, mycophenolate and corticosteroids.
- **Arm 2 (investigational therapy):** reduced-exposure tacrolimus, conversion of mycophenolate to Zortress® (everolimus) at 3 month post-transplant and corticosteroids.

In both study groups, either basiliximab 20 mg IV will be administered on post-operative day (POD) 0 and 4, or rabbit anti-thymocyte globulin 1.5 mg/kg on POD 0, 1, 2, 4 and 5. Choice

of induction will be based on the patient's immunologic risk (PRA) or risk of developing DGF. Tacrolimus doses will be adjusted to obtain target blood concentrations 5-12 ng/mL (using LCMS) for the first 3 months post-transplant. In the investigational arm, tacrolimus will be adjusted to obtain target trough concentrations of 2-5 ng/ml after 3 months post-transplant, once the patient is transitioned from mycophenolate to everolimus. The goal everolimus trough concentrations in this group will be 3-8 ng/mL (using LCMS). In the standard arm, tacrolimus doses will be adjusted to maintain a trough concentration of 5-12 ng/mL throughout the study period. Dose adjustments for immunosuppression will be allowed based on side effects or toxicities, at the clinical judgment of the primary investigator. Trough monitoring of mycophenolic acid will not be performed unless clinically warranted and dosage adjustments from such levels will be made only with consent of the study primary investigator. In the investigational arm, everolimus will be initiated at month 3 post-transplant at a dose of 1.25 mg twice daily in African-Americans and 1 mg twice daily in non-African-Americans. Doses will be adjusted to maintain trough concentrations of 3-8 ng/ml. Corticosteroids will be based on institutional protocol and will be continued in all patients at a minimum dose of 5 mg daily of prednisone or equal potent exposure if a different corticosteroid is utilized. Antimicrobial and anti-fungal prophylaxis will be administered based on institutional protocol.

Whole blood trough level monitoring of tacrolimus will be performed as follows, at a minimum of: post-operative day 3, 5, and 7, weeks 2, 3, 4, and months 2, 3, 6, 9 and 12. Everolimus whole blood trough level monitoring will be performed as follows, at a minimum of: post everolimus conversion day 4, weeks 1, 2, 3, 4 and months 3, 6, and 9. Tacrolimus and everolimus dosing may be adjusted as medically required for toxicity or rejection.

Study duration: 42 months total, with 6 months for start-up and initiation, 18 months for patient recruitment and randomization, 12 months of patient follow-up, and 6 months for data analysis and final report/manuscript preparation.

Study design: Prospective, randomized, comparative efficacy, open label study.

Study Procedures and Definitions:

A. Supplementary medications:

Patients will receive anti-infectives as per post-transplant protocol regimens as per usual care. Anti-infective prophylaxis: prophylactic antifungal therapy will consist of Nystatin 5 mL swish and swallow by mouth four times daily for 30 days post-transplant. Alternative therapies may be utilized based upon patient tolerability. Patients will receive PJP prophylaxis with sulfamethoxazole/trimethoprim single-strength tablet once daily for 6 months post-transplant. Sulfa allergic patients may receive either inhaled Pentamidine or oral daily Dapsone therapy depending on patient and physician preference. All patients at moderate (R+) or high-risk (D+/R-) for CMV infection will receive oral valganciclovir therapy for 3 to 6 months post-transplant.

B. Clinical and Laboratory Assessments:

Laboratory assessments: Laboratory evaluations will be performed at certified laboratories according to the study schedule. Required tests are specified in the study flow chart (see Appendix I).

Clinical assessments: All patients will undergo clinical evaluation by the investigator or the investigator's designee at the times specified in the study flow chart (see Appendix I). Clinical evaluations will include assessments of sitting blood pressure, patient weight changes, GI symptoms assessment, serious adverse events, study drug doses and concentrations, allograft rejection, infection, need for dialysis, hospitalizations; re-transplant status, and other immunosuppressant medications.

Graft function assessment**:** To monitor graft function, serum creatinine will be assessed immediately post-operatively to establish initial renal function. Creatinine clearance will be assessed at 1 week, 3 months, 6 months and 12 months using the abbreviated MDRD equation.

Acute renal dysfunction: renal dysfunction will be monitored by serial measurements of serum creatinine. All episodes of renal dysfunction defined as an incremental increase in serum creatinine concentration 20% above baseline or ≥0.4 mg/dL will be evaluated for rejection by renal biopsy and ultrasound, after the exclusion of other causes renal graft dysfunction (such as infection, dehydration and CNI toxicity).

C. Definition and diagnosis of rejection episodes:

Acute Rejection is defined as: elevation of serum creatinine of at least 20% above baseline or an absolute increase of greater than 0.4 mg/dl with renal allograft biopsy showing grade ≥1A rejection by Banff criteria.

Renal biopsy should be performed in all cases of renal dysfunction (after exclusion of causes other than rejection), to confirm a rejection episode. All patients must have biopsy confirmation of rejection episodes either before or within 24 hours of onset of treatment for rejection.

Rejection reversal is defined as return of serum creatinine to within 10% of baseline and/or histologic clearance by Banff criteria.

Resistant acute rejection is defined as no histologic improvement documented by renal biopsy within 7-10 days of initiation of treatment.

Recurrent acute rejection is defined as rejection recurring more than two weeks after documented rejection reversal.

Renal function will also be assessed by an estimated creatinine clearance utilizing the abbreviated MDRD equation.

D. Treatment of acute rejection episodes:

Acute rejection should be treated according to the following chart: Biopsy must be performed within 24 hours of suspected acute rejection.

Borderline and Banff '97 1A		
First line	Methylprednisolone 500 mg IV daily	X 3 doses
• Consider re-biopsy in 7 days if inadequate response to therapy • Increase maintenance immunosuppression and levels to upper limit of target range		
Banff '97 1B or Greater		
First line	Rabbit anti-thymocyte globulin 1.5 mg/kg/day, methylprednisolone 500 mg x 1 prior to first dose of Rabbit anti-thymocyte globulin	x 10 doses *or* 10 days of total suppression; ensure FK ≥8 ng/mL at time of RATG discontinuation
Second Line	Alemtuzumab 30 mg IV x 1 dose, methylprednisolone 500 mg x 1 prior to dose	x 1 dose
• No change in mycophenolate mofetil dose while receiving treatment for acute rejection • Consider re-biopsy at the end of acute rejection therapy		
Patients who receive T-cell depleting therapy must be started on valganciclovir and anti-microbial prophylaxis for three months following treatment of rejection		

E. Cardiovascular Disease Risk Factors

Hypertension: Hypertension will be defined and treated according to Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (see Appendix). Seated blood pressure should be taken at a resting state; patient should be seated for approximately 5 minutes.

Hyperlipidemia: Hyperlipidemia will be defined and treated according to Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults guidelines.

New Onset Diabetes After Transplant (NODAT) : Post-transplant diabetes mellitus is defined by the new requirement for therapy with insulin for ≥ 30 days or the use of oral hypoglycemic agents ≥ 30 days and current ADA requirements for the diagnosis of diabetes mellitus. Periodic assessments of blood glucose control will be measured by HbA1c levels when deemed clinically relevant.

F. Infection:

Infection is defined as by the following:

1) Treatment with antimicrobial agent for a specific clinical syndrome (not prophylaxis) AND positive cultures, pathologic identification of microbial agents, or significant serologic changes related to clinical symptoms
2) CMV infection is defined as the presence of CMV DNA is the patient's serum (CMV PCR) of at least 500 IU/mL on at least two separate occasions without clinical signs or symptoms of infection.
3) Significant CMV infection is defined as the presence of CMV DNA is the patient's serum (CMV PCR) of at least 10,000 IU/mL on at least two separate occasions without clinical signs or symptoms of infection.
4) CMV syndrome is defined as a CMV infection with clinical symptoms (malaise, diarrhea, weakness, anorexia, and/or N/V) with clinical signs (fever, leukopenia)
5) CMV disease is defined as invasive or symptomatic CMV infection with histological evidence of viral cytopathic effect or a positive CMV culture from a deep tissue specimen in the setting of suggestive clinical manifestations. Specimens used for diagnosis of CMV disease include kidney, liver or lung biopsy, endoscopic mucosal biopsy or brushing, bronchoscopic mucosal biopsy or brushing, bronchoalveolar lavage, and cerebrospinal fluid.
6) BK infection is defined as the presence of BK DNA is the patient's serum (BK PCR) of at least 500 IU/mL on at least two separate occasions.
7) Significant BK infection is defined as the presence of BK DNA is the patient's serum (BK PCR) of at least 10,000 IU/mL on at least two separate occasions.
8) BK nephropathy is defined as the presence of BK DNA is the patient's serum (BK PCR) of at least 500 IU/mL on at least two separate occasions AND the presence of graft dysfunction in the presence of a histologic diagnosis of the kidney allograft that is consistent with BK nephropathy per current Banff criteria.
9) Other opportunistic viral infections: These will be defined as the presence of clinical signs and symptoms of infection consistent with that particular viral pathogen AND the presence of viral DNA in the patient's serum. These viruses include Herpes Simplex, Varicella Zoster, Epstein Barr, Parvovirus, and Adenovirus.
10) Clostridium difficile associated colitis will be defined by the presence of signs (fever and leukocytosis) and symptoms (diarrhea, N/V, malaise, anorexia) of C dif AND a positive C dif screen by either toxin or PCR detection.

G. **Gastrointestinal toxicity**:

Gastrointestional toxicities including nausea, vomiting, and diarrhea will be monitored for and recorded. If clinically significant GI toxicities occur in a study patient, immunosuppressant medications known to induce these adverse effects (including tacrolimus, mycophenolate and everolimus) may be reduced in dose, temporarily held or permanently discontinued. The decision to alter immunosuppression should be made on the severity of the toxicity and the patients overall clinical scenario. Such decisions will be made by the study principle investigator.

H. **Malignancies:**

Development of any post-transplant malignancies will be evaluated and recorded up to one year post-transplant. All malignancies, including post-transplant lymphoproliferative disorder (PTLD) will be defined by current American Cancer Society diagnostic criteria. Immunosuppression regimens may be reduced, temporarily held or permanently discontinued as per usual care if a patient develops post-transplant malignancy. Such decisions will be made by the study principle investigator in consultation with the patient's oncologist.

I. Immunosuppressant Side Effects:

Immunosuppression adverse effects will be assessed at each clinic visit. The criteria to determine the incidence and severity of immunosuppression adverse events will occur using the Common Toxicity Criteria for Adverse Events version 2.0 (CTCAE). Immunosuppression regimens may be reduced, temporarily held or permanently discontinued as per usual care if a patient develops immunosuppression induced adverse events. Such decisions will be made by the study principle investigator.

J. Gene variant analysis:

Genotyping of the SNPs listed in the table below will be performed using next generation sequencing technology, which is emerging as a rapid and cost effective alternative to commonly used SNP assays such as Taqman SNP and PCR-RFLP assays. This work will be performed in the MUSC Proteogenomics Facility, which operates an Ion Torrent PGM (Life Technologies, Grand Island, NY). Primer pairs flanking the polymorphic positions will be synthesized such that amplicons are 150-300bp with SNPs centrally located. Multiplex PCR will be performed for each patient sample using the primer pairs in amplification reactions on a Cfx-96 real time PCR instrument (Bio-Rad, Hercules CA). Secondary amplifications will be performed using a second series of primers modified to contain 8-nt barcodes (on the 5' end of the primer) that are unique for each patient. Sequencing adaptors will be ligated onto the purified amplicons using the Ion Plus Fragment Library Kit (Life Technologies), and the products will be subjected to 200bp template preparation and sequencing on Ion 314 chips (approximately 10 Mb sequence output) according to Ion Torrent recommendations. Genomic DNA samples will be processed in batches of 30, with average sequence length of 200bp, the 30 genomic samples can be sequenced on the same 314 chip to achieve an estimated coverage of 100X. Ion sequences will be trimmed, filtered, assigned based on barcode, and aligned to the human genome using Ion TMAP software. SNP calling will be done on aligned sequence (BAM file) with the Ion DiBayes SNP Detection algorithm run in the "frequentist" configuration.

Protein	SNP(s)	Association with Clinical Outcomes
P-Glycoprotein (PGP, ABCB1, MDR1)	G2677T/A, C3435T, C1236T	• FK and CyA Nephrotoxicity • FK Neurotoxicity in Liver Transplant Recipients • CNI Neurotoxicity in Stem Cell Transplants • CyA DGF, Renal Function, NODAT, & CMV • FK Nephrotoxicity in Pediatric Liver Transplant • Increased Risk of Acute Rejection with CyA
Apolipoprotein L1 (APOL1)	G1 and G2	• Kidney Disease and Kidney Failure

K. Graft Interstitial Fibrosis:

Images will be examined with a Nikon E600 microscope, and a Spot Digital Firewire camera will be used to capture 24 bit RGB color images that will be stored as TIFF files. A background image of a blank area of the slide will be initially obtained and background correction will be performed in real time to adjust for subtle irregularities in the illumination of the microscope field. The images will be acquired using the 20X objective with a numerical aperture of 0.5. Images of the cortex of the biopsy from one end to the other will be obtained in a serpentine fashion starting at one end of the tissue and working toward the other. Image analysis will be performed on the stored images using an automated macro specially written for the software package NIH ImageJ. Automated analysis of the images will be performed with operator supervision. After the software is set to differentiate the positively stained from negatively stained areas on the first image, the software will sequentially open each image, do the analysis, store the data, close the image, and move on to the next image until the entire biopsy is analyzed. The operator's only function during the analysis phase is to watch the screen and stop the process if an error is detected.

L. Donor Specific Antibodies:

Serum samples will be collected and stored at the time of each renal allograft biopsy (both protocol based and for cause). DSA screening will be performed using FlowPRA beads representing HLA-A, -B, -Cw, -DR, -DQ and -DP antigens (One Lambda, Canoga Park, CA, USA). If the screening assay is positive, determination of HLA antibody specificities will be performed using FlowPRA single antigen class I and II beads (One Lambda) and analyzed according to the manufacture's recommendations. HLA antibody specificities will be validated using LABScreen single antigen beads using a threshold mean fluorescence intensity ≥300 (One Lambda).

M. Gene Expression:

All renal allograft biopsies (both protocol based and for cause) will have core tissue immediately stabilized in RNAlater® to prevent mRNA degradation. These samples will be stored at -70º C until processing. Absolute levels of mRNA will be quantified using preamplification-enhanced real-time quantitative polymerase-chain reaction

(PCR) assays with the use of oligonucleotide primers and TaqMan probes. The specific mRNA that is quantified will be determined by study investigators at the time of analysis, as this is a rapidly changing field and new mRNA targets may be found while the study is recruiting. At this time, mRNA targets are proposed to include CD3ε, perforin, granzyme B, proteinase inhibitor 9, CD103, IP-10, CXCR3, and transforming growth factor β1 (TGF-β1).

N. Urinary Proteomics

These analyses will be conducted using the algorithms developed by Arthur and Janech. Urine samples will be stored at -80 degree C for proteomic analysis. Proteins will be denatured, alkylated and digested with trypsin. Each sample will be pre fractionated using offline reversed phase solid phase extraction. Solid phase extraction fractions will be analyzed using LC-MS/MS. Tandem mass spectrometry will be performed using and AB SCIEX (Framingham MA) Triple TOF 5600 mass spectrometer. The Scaffold unweighted spectral counts of identified proteins will be normalized to internal standard protein in each sample and the relative abundance of each protein will be reported in normalized spectral counts.

O. Pulse Wave Velocity and Augmentation Index

Measurements of pulse wave velocity and vascular stiffness will occur at prespecified time points as delineated in the Study Evaluation Schedule located in the Appendix at the end of this protocol (baseline, month 3 and month 12 post-transplant). The following procedure will be used to obtain each of these measurements: After participants have rested for at least 10 minutes in the supine position, arterial waveforms at the radial arm (not containing vascular access grafts or fistulas), carotid, or femoral artery pulses will be recorded by applanation tonometry. Applanation tonometry will be performed by placing a micromanometer-tipped probe against the skin surface over the artery of interest and obtaining pressure waveforms transmitted through the skin surface. Pressure waveforms will be recorded during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, TX, USA) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). To determine aortic BP, the pulse wave obtained from radial tonometer recordings will be calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The radial pressure waveform will be converted into a central (aortic) waveform using a validated generalized transfer function (GTF) incorporated in SphygmoCor software. Central aortic systolic and diastolic BP will be derived from the aortic waveform and PPc will be calculated from the difference.

Safety Assessment Definitions:

A. Renal Function:

Renal function will be calculated by the eGFR using the 4-variable MDRD formula. Time points for calculations are delineated in the Study Evaluation Schedule located in the Appendix at the end of this protocol. The primary renal function assessments utilized for safety and efficacy analyses will be the eGFR at baseline (randomization) and at 12 months post-transplant.

B. Physical Examination:

A thorough physical assessment will be performed at screening/randomization and at months 3, 4, 6 and 12 post-transplant. Significant findings that are present prior to the start of the study will be included in the Relevant Medical History/Current Medical Conditions on the CRF. Significant findings made after the start of study medication which meet the definition of an AE will be recorded in the AE CRF.

C. Vital Signs:

Vital signs (radial pulse rate and blood pressure) will be recorded as indicated in the the Study Evaluation Schedule located in the Appendix at the end of this protocol. Blood pressure and pulse rate will be assessed at the same arm each time of determination and after the subject has rested in the sitting position (may be supine if during hospitalization) for at least five minutes. Measurements will be recorded on the CRF.

D. Height and Weight:

Height and weight will be measured and recorded at screening and during follow-up visits when physical exams are performed. Results will be recorded on the CRF.

E. Laboratory Evaluations:

All laboratory measurements will be performed at MUSC or referral centers that are routinely used as part of usual follow-up care. Specific information regarding the types and schedule of laboratory assessments can be found in the Study Evaluation Schedule located in the Appendix at the end of this protocol. Laboratory measurement results and date of collection will be recorded for the baseline and follow-up time points within the CRFs. Sample collection and analysis for the proteomics, genomics and gene expression analyses will be conducted as described in the above sections.

F. Pregnancy, contraception and assessment of fertility:

Pregnancy testing (serum) will be carried out for all females of child-bearing potential as described in in the Appendix at the end of this protocol. Testing will be part of routine follow-up care and will be conducted pre-transplant, within 7 days of transplant and at months 6 and 12 post-transplant.

Females of reproductive potential taking mycophenolate must receive contraceptive counseling and use acceptable contraception as delineated below for the entire time while on mycophenolate and for 6 weeks after stopping mycophenolate, unless the patient chooses abstinence (she chooses to avoid heterosexual intercourse completely). Females of reproductive potential taking everolimus must receive contraceptive counseling and use acceptable contraception as delineated below for the entire time while on everolimus and for 8 weeks after stopping everolimus, unless the patient chooses abstinence (she chooses to avoid heterosexual intercourse completely).

Acceptable contraception methods for females of reproductive potential

Option 1 (monotherapy methods):

1. Intrauterine devices (IUDs)
2. Tubal sterilization
3. Patient's partner had a vasectomy

Option 2 (Choice one from category A AND one from category B):

1. Category A:
    a. Estrogen and progesterone
        i. Oral contraceptive pill
        ii. Transdermal patch
        iii. Vaginal ring
    b. Progesterone only
        i. Injection
        ii. Implant
    c. Barrier method
        i. Diaphragm with spermicide
        ii. Cervical cap with spermicide
        iii. Contraceptive sponge
2. Category B:
    a. Male condom
    b. Female condom
    c. Diaphragm with spermicide (for use with hormone therapy)
    d. Cervical cap with spermicide (for use with hormone therapy)
    e. Contraceptive sponge (for use with hormone therapy)

G. Choice of Safety measurements:

The safety laboratory assessments (renal function, chemistry, hematology) selected are standard for the kidney transplant population. The blood levels of everolimus and tacrolimus are required for correct control of drug levels and dosing. The assessment of (serious) adverse events, including infections, graft loss, death, dialysis and details of certain viral infections (CMV and BKV) allow for proper assessments of safety related outcomes and side effects in this population.

Safety Monitoring:

A. Adverse Events:

An adverse event (AE) is any untoward medical occurrence (i.e., any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a subject or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product. The occurrence of adverse events should be sought by non-directive questioning of the subject at each visit during the study. Adverse events also may be detected when they are volunteered by the subject during or between visits or through physical examination, laboratory test, or other assessments. Abnormal laboratory values or test results constitute adverse events only if they fulfill at least one of the following criteria:

- they induce clinical signs or symptoms,
- they are considered clinically significant,
- they require therapy.

Clinically significant abnormal laboratory values or test results should be identified through a review of values outside of normal ranges/clinically notable ranges, significant changes from baseline or the previous visit, or values which are considered to be non-typical in subject with underlying disease. Investigators have the responsibility for managing the safety of individual subject and identifying adverse events. Adverse events should be recorded in the Adverse Events CRF with them accompanied by the following information:

- the severity grade: (mild: usually transient in nature and generally not interfering with normal activities, moderate: sufficiently discomforting to interfere with normal activities, severe: prevents normal activities)
- possibility of relationship to the study treatment (no/yes)
- its duration (start and end dates) or if the event is ongoing an outcome of not recovered or not resolved should be reported.
- whether it constitutes a serious adverse event (SAE), if so report date should be provided
- action taken regarding study treatment
- whether other medication or therapies have been taken (concomitant medication/non-drug therapy)
- its outcome (not recovered/not resolved; recovered/resolved; recovering/resolving, recovered/resolved with sequelae; fatal; or unknown)

All adverse events should be treated appropriately by the PI or designee. Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary).

B. Serious Adverse Events:

An SAE is any adverse event which meets any one of the following criteria:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity

- constitutes a congenital anomaly/birth defect
- leads to a hospitalization that is not related to a renal allograft biopsy, renal allograft rejection episode or infection

AEs and SAEs will be reported in accordance with the FDA Guidelines for Post-Marketing Reporting of Adverse Events 21 CRF 314.80 and 21 CRF 600.80 **http://www.fda.gov/medwatch/report/regs.htm**. Any serious adverse event or a non-serious adverse event that is related to the study and unexpected will be reported to the FDA, per reporting guidelines, as soon as possible, but no later than 15 working days. Any serious adverse event or a non-serious adverse event that is related to the study and unexpected will be reported to Novartis within 24 hours of occurrence. Further, all serious adverse events that are related to the study and are unexpected must be reported to The Medical University of South Carolina IRB within 10 days per The Medical University of South Carolina IRB Unanticipated Problems and Adverse Events Policy and Procedures reporting guidelines.

C. Pregnancy Reporting:

Any pregnancies occurring while the subject is on study treatment will be reported to Novartis within 24 hr of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Statistical analysis:

Categorical data will be evaluated based on Fisher's Exact and Chi Square tests where appropriate; descriptive statistics will be used for continuous variables to describe the two patient populations and primary outcomes in each group (% change in interstitial fibrosis and eGFR from month 3 to month 12). Student's T-test or comparable non-parametric tests (such as the Wilcoxon Rank Sum test) will be used to compare the groups for these continuous variables. Patients will be analyzed based on an intent-to-treat basis, except when prohibited by lack of follow-up 12 month biopsies. Kaplan-Meier curves with the log rank test will be used to compare patient and graft survival, acute rejection free survival, and *de novo* donor specific antibody free survival. A p-value of <0.05 will be considered statistically significant. Slope change of eGFR from 3 months to 12 months post-transplant will be calculated and compared as well.

For the genomic comparisons, patients will be grouped based on the presence or absence of known single nucleotide polymorphisms and compared for primary outcomes. If significant baseline differences are noted between groups, multivariate analysis will be conducted to control for these differences. Similar analyses will be conducted for change in mRNA expression and change in urinary proteomic excretion between month 3 and month 12.

This will be an intent-to-treat analysis with all patients undergoing randomization included in the final data analysis, even if removed from study or control medication. Patients screened and consented, but not randomized will be tracked for reasons of non-randomization, but will not be followed for clinical outcomes or included in the final analysis. These patients will be

replaced to insure a total of 60 patients (30 in each group) are randomized and followed to meet study power and sample-size.

Sample size and power: A total of 60 patients, 30 *randomized* in each arm, will provide 89% power (alpha=0.05, two-sided) to detect a significant difference in the percent change in the quantity of interstitial fibrosis between groups when comparing the three month and 12-month protocol biopsies. These calculations used the following assumptions: 10% drop out rate for each group (27 patients in each group will have baseline and follow-up biopsies), with a 64±41% change in degree of fibrosis in the control arm and a 13±71% change in fibrosis in the investigational arm.

Evaluation schedule:

Assessments		Pre-Txp	Day Post-transplant							Week Post-Txp		Month Post-transplant			
Time Frame			1	2	3	4	5	6	7	2	4	3	4	6	12
RANDOMIZATION												X (10-16 week window)			
History and Physical[2]		X							X	X	X	X	X	X	X
Vital Signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Protocol Kidney Biopsy												X			X
Donor Specific Antibody Analysis												X			X
Pregnancy testing[7]		X	X (anytime between day 1-7)											X	X
Serologies[1]		X													
Laboratory Assessment	CBC and Chem-7[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Total cholesterol, HDL, LDL, triglycerides[3]	X									X	X			X
	Spot Urine Protein/ Creatinine Ratio										X	X		X	X
	Tacrolimus levels[5]				X	X	X	X	X	X	X	X	X	X	X
	Everolimus levels											X	X	X	X
Clinical assessment[6]			X	X	X	X			X	X	X	X		x	X
EKG		X													
Estimated GFR		X	X	X	X	X	X	X	X	X	X	X		x	X
Genomic, RNA and proteomic analysis[4]												X			X
Applanation tonometry using pulse wave velocity and augmentation index		One Baseline Measurement										X			X

Foot notes:

1. Includes testing for CMV, Epstein-Barr, hepatitis B & C and HIV for donor and recipients (from evaluation or within one year pre-transplant if the results were negative at pre-transplant evaluation)
2. Within 48 hours prior to transplant
3. Fasting for at least 8 hours
4. Includes genetic SNP analysis, mRNA expression, and urinary proteomic analysis
5. Tacrolimus concentrations will be measured daily during the first week post-transplant once tacrolimus is initiated
6. Includes assessments of blood pressure, weight changes, arterial stiffness measurement, adverse events, study drug doses, allograft rejection, infection, need for dialysis, hospitalization, re-transplant status, and other immunosuppressant medications
7. Includes pregnancy test for females of child-bearing potential

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