PROTOCOL TITLE:

ONCE-DAILY EXTENDED-RELEASE TACROLIMUS VS. TWICE-DAILY TACROLIMUS: IMPACT ON T-CELL SUBPOPULATIONS AND MARKERS OF RENAL TUBULE-TOXICITY IN KIDNEY TRANSPLANT PATIENTS

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OBJECTIVES AND HYPOTHESIS:

The overall aim of the present study is to prospectively investigate the impact of two maintenance CNI immunosuppressive regimens (both prednisone-free, with MMF) on subpopulations of T and B cells and alloreactive T cells as well as on renal allograft function at week 2, 3 months and 12 months post-transplantation.

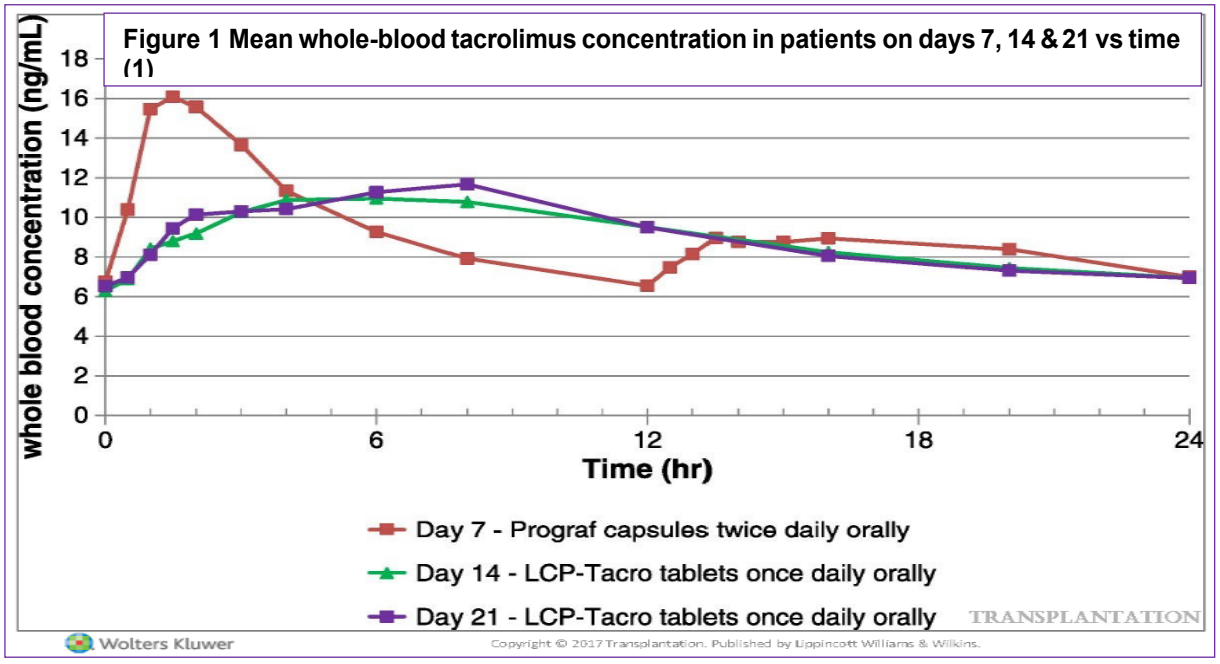
1. To compare renal toxicity of once-daily LCP-Tacro/Envarsus XR vs. twice/day Tacrolimus by measured acute kidney injury markers.
2. To compare the effect on T and B cell subpopulations of once-daily LCP-Tacro/Envarsus XR vs. twice /day Tacrolimus. Specific studies to include prospective, serial flow cytometric analysis of T and B cell subpopulations, as well as high throughput sequencing T cell receptor repertoire analysis monitoring for presence or absence of donor-reactive T cells.

Our hypothesis is that that more consistent exposure with LCP-Tacro/Envarsus® XR avoiding under immunosuppression will prevent the emergence of alloreactive T cells, development of effector memory T cells, and a reduced incidence of immunologic allograft injury. We also posit that avoidance of the peaks associated with conventional twice daily tacrolimus will spare the renal allograft from drug induced nephrotoxicity.

BACKGROUND:

Kidney transplantation is the treatment of choice for most patients with end-stage renal disease. Lifelong immunosuppressive therapies are required to prevent organ rejection. However, long term exposure to immunosuppressive therapy after kidney transplantation can place patients at risk for multiple adverse events. The optimal immunosuppressive therapy is not well established. Tacrolimus, a calcineurin inhibitor (CNI) is highly effective in preventing acute rejection after organ transplantation (2). It is used as part of the immunosuppression regimen for the majority of kidney and liver transplant recipients (3). However, treatment with current formulation of Tacrolimus generates high peaks and low troughs in drug concentrations in the blood. It is known that high exposure to CNI is associated with renal toxicities and adverse events (4). New once-daily dosage formulations are now developed with the hope of minimizing side effects while maintaining excellent outcomes (5-8).

LCP-Tacro/Envarsus® XR, a new once-daily formulation of tacrolimus, was approved by the



FDA in 2015 for conversion from twice-daily tacrolimus in kidney transplant recipients. It is a prolonged-release tacrolimus formulation, utilizing a MeltDose drug delivery technology designed to improve the bioavailability of drugs with low water solubility (1). Recent clinical data demonstrated that once-daily LCP-Tacro/Envarsus XR has improved pharmacokinetic bioavailability, rapid achievement of therapeutic trough levels, less fluctuation and swing in whole blood concentration, non-inferior efficacy and similar safety, with lower tacrolimus dose than other tacrolimus formulations (1, 9-13). (**Figure1**).

INCLUSION AND EXCLUSION CRITERIA:

The target population is adult recipients of immediately functioning living and deceased donor renal allografts. Immediate function will be defined as the absence of the need for hemodialysis in the first week following renal transplantation.

Inclusion Criteria:

1. Patients who are males or females aged 18-65 years.
2. Donors aged 18-65 years.
3. No prior organ transplant
4. Patients who are single-organ recipients (kidney only).
5. Women who are of childbearing potential must have a negative serum pregnancy test before transplantation and agree to use a medically acceptable method of contraception throughout the treatment period.
6. Subject (recipient) is able to understand the consent form and give written informed consent

Exclusion Criteria

1. Delayed graft function (please see above).
2. Known sensitivity or contraindication to alemtuzumab, Envarsus® XR, tacrolimus or MMF.
3. Use of the following induction medications: basiliximab and rituximab.
4. Patient with significant or active infection.
5. Patients with a positive flow cytometric crossmatch using donor lymphocytes and recipient serum.
6. Patients with PRA > 40%
7. Patients with current or historic donor specific antibodies
8. Body Mass Index (BMI) of < 18 or > 35
9. Patients who are pregnant or nursing mothers.
10. Patients whose life expectancy is severely limited by diseases other than renal disease.
11. Ongoing active substance abuse, drug or alcohol.
12. Major ongoing psychiatric illness or recent history of noncompliance.
13. Significant cardiovascular disease (e.g.):
    a. Significant non-correctable coronary artery disease;
    b. Ejection fraction below 30%;
    c. History of recent myocardial infarction.
14. Malignancy within 3 years, excluding non-melanoma skin cancers.
15. Serologic evidence of infection with HIV or HBVs-Ag positive.
16. Patients with a screening/baseline total white blood cell count < 4,000/mm3; platelet count < 100,000/mm3; triglyceride > 400 mg/dl; total cholesterol > 300 mg/dl.
17. Investigational drug within 30 days prior to transplant surgery.
18. Anti-T cell therapy within 30 days prior to transplant surgery.

19. Diagnosis of atypical-Hemolytic Uremic Syndrome (aHUS).
20. Subjects transplanted with a Hepatitis C NAT-positive kidney.

NUMBER OF PARTICIPANTS:

Prospective randomized single center open label study of 2 groups of kidney transplant patients

- Group 1 : standard of care (SOC) control group will receive tacrolimus twice-daily (n=25)
- Group 2 : LCP-Tacro/Envarsus® XR group will receive LCPT tablets once daily (n=25)

RECRUITMENT METHODS:

The investigators will recruit patients in a manner that is sensitive to the inclusion of women and members of minority groups into this study. The renal failure population awaiting transplantation on the participating program waiting lists is representative of the renal failure population in general. Subjects will be recruited from the patients who present themselves to Northwestern University, Kovler Organ Transplantation Clinic, located in Arkes Pavilion, 676 N. St. Clair Street, Suite 1900, Chicago, IL 60611.

Subject Information and Consent

Prior to any testing under this protocol, including screening tests and evaluations, written informed consent will be obtained from the subject in accordance with local practice and regulations. Whenever possible, the investigator(s) will also be involved in this procedure. The background of the proposed study and the benefits and risks of the procedures and study will be explained to the subject. A copy of the informed consent document signed and dated by the subject will be given to the subject. Confirmation of a subject's informed consent will be documented in the subject's medical records prior to any testing under this protocol.

Avoidance of Coercion

It will be made clear to all patients that they can receive transplantation therapy (contingent on their medical suitability) without participation in this trial.

STUDY TIMELINES:

We expect the total duration for this study not to exceed 2 years from the date of the last subject's enrollment. Each individual subject will be part of the study for 12 months after transplantation.

STUDY ENDPOINTS:

Primary endpoints:

One of the 2 primary end points Kidney transplant function assessed as kidney injury markers in urine samples collected at 12-hour and 24-hour trough levels and 2-hour peak levels at 2nd week, 3rd month,12th month post transplantation, and at any for-cause biopsy event. The injury markers will be assessed using Myriad-RBM KidneyMAP panel https://myriadrbm.com/products-services/humanmap-services/kidneymap/.

The other primary end point is immune activation assessed through serial flow cytometric immunophenotyping for immune cells including T and B cell subpopulations as well as donor-reactive TCR repertoire analysis in biopsy, blood and urine.

Secondary endpoints:

Kidney biopsy will be performed per SOC at month 3 and 12 post-transplant. Biopsies will be evaluated and compared between two groups for CNI-related toxicities. Correlations

between kidney injury markers and biopsy data at 3 and 12 months post-transplant will be performed (details are below).

Antibody responses and correlation with CNI exposure by SOC donor specific antibody (DSA) assessment at months 3 and 12 post-transplant (performed at the Clinical Immunology Laboratory of the CTC).

PROCEDURES INVOLVED:

Study Design:

Donors: Blood samples (50ml) from donor subjects will be obtained before transplantation. These donor leukocytes will be used as stimulator cells in studies of functional activity of recipient T-cells. These samples will be processed in the research laboratory of co-investigator, Dr. James Mathew.

Renal Transplant Recipients: All recipients will receive the same induction immunotherapy at the time of transplant.

Mycophenolate (MMF, Myfortic®)
The MPA/Myfortic will be initiated at 720- 1250 mg BID orally. The first dose will be given, per SOC. The agent will be given in an open label fashion. Once discharged from the hospital, the subject will obtain this medication via a prescription at a pharmacy of their choice.

Alemtuzumab (Campath-1H®)
One dose will be 30mg given IV, and administered intra-operatively during renal transplantation. The agent will be given in an open label fashion and will only be administered in the hospital.

Corticosteroids
Intravenous corticosteroids (prednisolone, Solumedrol®)

Time: Dose
Preop (Intraop) 500mg
POD#1 250mg
POD#2 125mg

No further steroids will be given post-transplant unless indicated by the following medical conditions: acute renal allograft rejection, renal diseases necessitating the use of steroids, and other systemic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and asthma.

Tacrolimus (Prograf®)
Tacrolimus will be given 1-2 mg BID, per SOC. The dose will be modified to achieve 12 hour trough concentrations of 8-12 ng/ml. The agent will be given in an open label fashion. Half of the study subjects (randomized; n=25) will be on this drug treatment

LCP-Tacrolimus/Envarsus® XR (Study related drug)
LCP-Tacro/Envarsus® XR a new once-daily formulation of tacrolimus, was approved by the FDA in 2015 for conversion from twice-daily tacrolimus (above) in kidney transplant recipients. It is a prolonged-release tacrolimus formulation, utilizing a MeltDose drug delivery technology designed to improve the bioavailability of drugs with low water solubility. The other half of the study subjects (randomized; n=25) will be on this drug treatment. LCP-Tacro/Envarsus XR will be given 5 mg QD to achieve a 24-hour trough concentration of 8-12 ng/ml.

Study Drugs
None of the drugs given in conjunction with this study are provided by the study sponsor, including LCP-tacro/Envarsus XR.

Recipient Samples collection:
Blood, urine and biopsies will be collected from the kidney transplant recipients as shown in **Figure 2** (bottom).

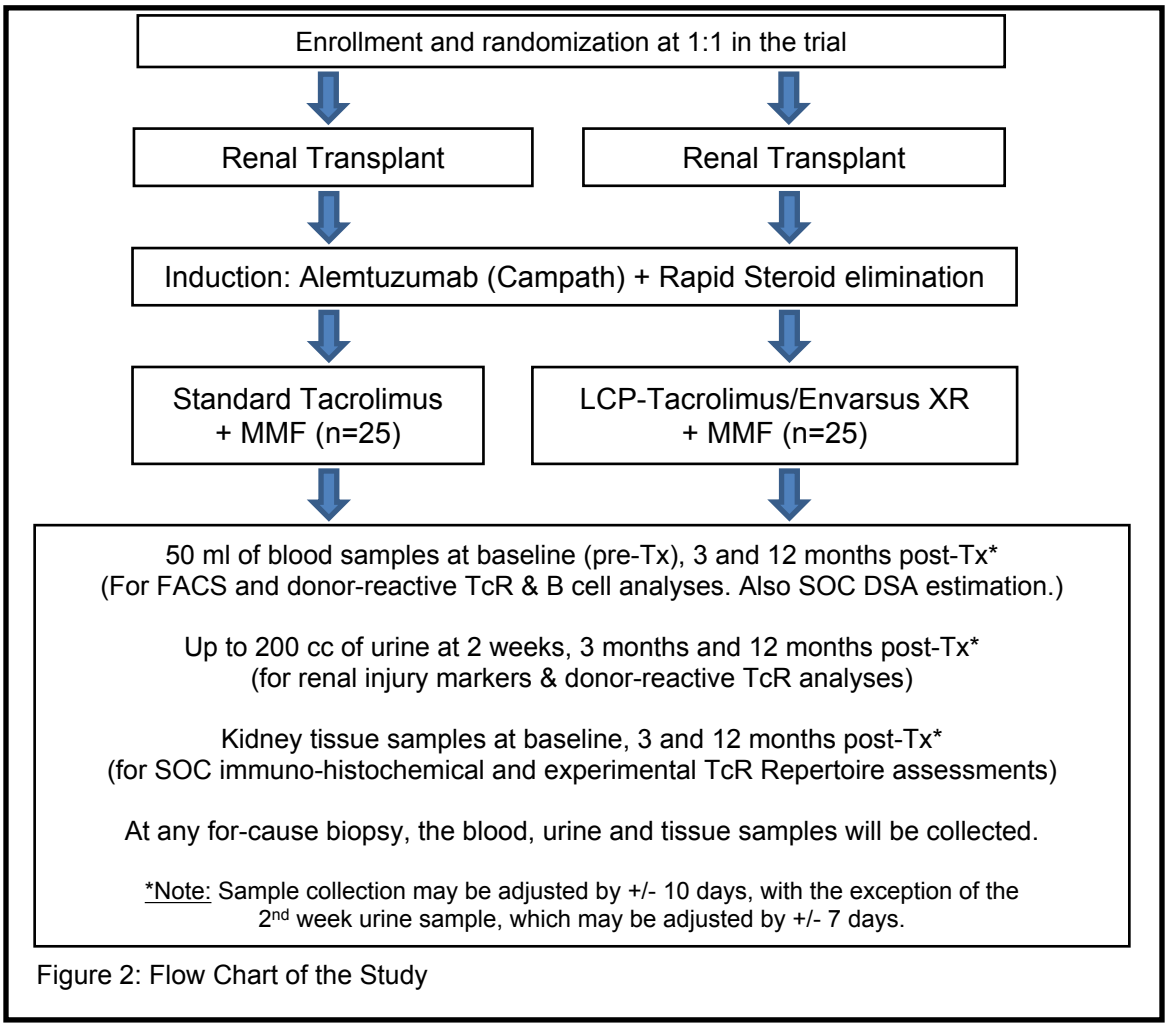


Figure 2: Flow Chart of the Study

Kidney transplant function:
For subjects receiving Standard Tacorolimus + MMF urine samples will be collected at 12-hour trough levels and 2-hour peak levels at 2nd week, 3rd month, 12th month post transplantation, and at any for-cause biopsy event.

For subjects receiving LCP-Tacrolimus/Envarsus XR + MMF, urine samples will be collected at 24-hour trough and 2-hour peak levels at 2nd week, 3rd month, 12th month post transplantation, and at any for-cause biopsy event.

Urine will be used for the analysis of kidney injury markers using Myriad-RBM KidneyMAP panel https://myriadrbm.com/products-services/humanmap-

services/kidneymap/. [The samples will be used also for TcR repertoire analysis; (please see below).]

Blood Immunophenotyping.
The approach will be to conduct a multi-parameter flow cytometric immunophenotyping of peripheral blood before and at 3 and 12 months post-transplant to analyze cellular changes.

Methods (14-16): Our well-established 5-color flow cytometric analyses is performed using whole blood to delineate the cellular makeup in terms of both percentages and absolute numbers (**Table 1**). RBCs are lysed before flow acquisition. The analysis is for defining the T cell subsets, monocytes and natural killer cells (Tube 2), the B cells

including naïve, transitional, memory and effector-memory subsets (Tube 3) and the dendritic cells including immature, monocytoid and plasmacytoid fractions (Tube 4).

Table 1: Whole blood immunophenotyping panel			
Tube 1	**Tube 2**	**Tube 3**	**Tube 4**
IgG-FITC	CD4-FITC	CD38-FITC	CD3, 14, 16, 19, 20, 56 FITC
IgG-PE	CD14-PE	IgD-PE & IgM-PE	CD33-PE
IgG-ECD	CD56-ECD	CD19-ECD	HLA-DR-ECD
IgG-PC5	CD3-PC5	CD27-PC5	CD11b-PC5
IgG-PC7	CD8-PC7	CD24-PC7	CD123-PC7

In addition to the whole blood analyses, PBMC are isolated from heparinized 40 cc blood samples by Ficoll-Hypaque gradients. Surface markers are identified with indicated monoclonal antibodies, and after fixation and permeabilization the intracellular protein ligands are detected with antibodies shown on the bottom row (**Table 2**). This panel delineates expression of markers for activated, effector, memory, anergic and exhausted cell fractions as well as myriad sub-sets of regulatory T and B cells.

Table 2: Immunophenotyping in using isolated PBMC (surface and intracellular)					
Tube 5	**Tube 6**	**Tube 7**	**Tube 8**	**Tube 9**	Staining
CD4-ECD	IgD/M-PE	CD3-ECD	CD49b FITC	KLRG-1 PE	Surface
CD25-PC5	CD19-ECD	CD28-PC5	LAG-3 PE	PD1-ECD	
CD127-PC7	CD27-PC5	CD8-PC7	CD4 ECD	CD3-PC5	
CTLA4-FITC	CD24-PC7	CD45RA -FITC		CD8 -PC7	
FoxP3-PE	IL-10 -FITC	FoxP3-PE	FoxP3 PC5 & IL10 PC7	Ki-67 FITC	Intra-cellular

Donor-reactive TCR repertoire analysis The approach will be to identify and estimate the total T cell clonal repertoire that a patient is capable of mounting against the donor by performing MLR and purifying reactive cells followed by ImmunoSEQ analysis (17) prior to transplant. Then after transplant, monitor for the absence, presence or amplification of the pre-identified T cell clones in the post-transplant biopsies, blood and/or urine samples. This is a novel innovative technology that we have developed for profiling recipient's immune status against the donor to correlate with the status of the graft. The methodological approach is follows:

Selection of donor-reactive T cells: Recipient pre-transplant peripheral blood mononuclear cells (PBMC) will be labeled with CFSE dye and then stimulated with PKH266-labled donor irradiated PBMC in MLR. After 7 days, the proliferating responder cells that have diluted the CFSE are flow-sorted (at NU Flow Core) as shown in **Figure 3** (top middle). Based on previous results, it is expected that the proliferating cells will be >90% $CD3^+$ T cells, distributed into $CD4^+$ or $CD8^+$ cells, with minimal contamination with $CD19^+$ B cells or $CD14^+$ monocytes but some $CD56^+$ NK cells. Both the $CD3^+CD4^+$ and

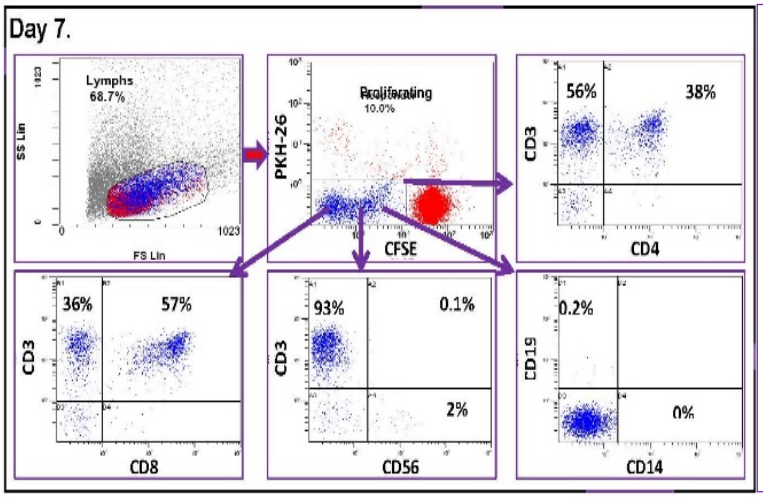


Figure 3. Flow Sorting and phenotypic subset analysis of donor reactive clones. Bulk MLRs were prepared and the cellular makeup of responder cell populations were delineated after 7 days in culture using fluorochrome coupled monoclonal antibodies. The cells were analyzed first by gating on lymphocytes and then after gating on CFSE diluted proliferating responder cells. The CFSE diluted donor reactive T cells were sorted for ImmunoSEQ analysis.

$CD3^+CD8^+$ cell subsets are sorted out separately and is shipped to Adaptive Biotechnologies in Seattle.

ImmunoSEQ analysis of donor-reactive T cells: At Adaptive Biotechnologies total genomic DNA is purified from the flow-sorted $CD3^+CD4^+$ and $CD3^+CD8^+$ cell subsets from each culture using Qiagen DNeasy Blood extraction Kit following the manufacturer's protocol. Then, a multiplex PCR assay that is capable of specifically amplifying rearranged TCRB genes from a complex background followed by deep sequencing is performed (**Figure 4**). The data is analyzed measuring clonality, proportion and diversity of the T cell repertoire (18-20). These represent the total T cell repertoire the recipient is capable of reacting to the donor. We have denoted the performance of the pre-transplant MLR, isolation of donor reactive clones and ImmunoSEQ analysis, collectively as **AlloSEQ**.

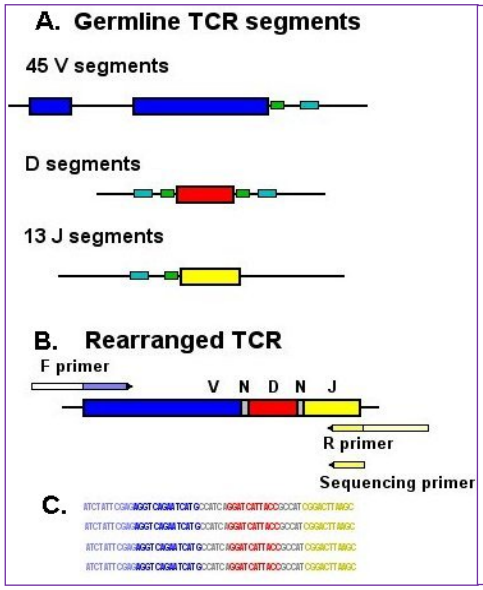


Figure 4. TCRβ sequencing assay. **(A)** The assay is designed to identify the V D and J segments in recombined CDR3 TCRβ genes **(B)** Forward primers were designed to amplify all 45 TCRβ V segments. Reverse primers were designed to each of the 13 TCRβ J segments. **(C)** Custom software tools have been developed to verify, collapse, align and catalog the CDR3 sequences for calculation of repertoire diversity.

Enumeration of donor reactive T cell clones in post-transplant kidney biopsies, blood and urine:

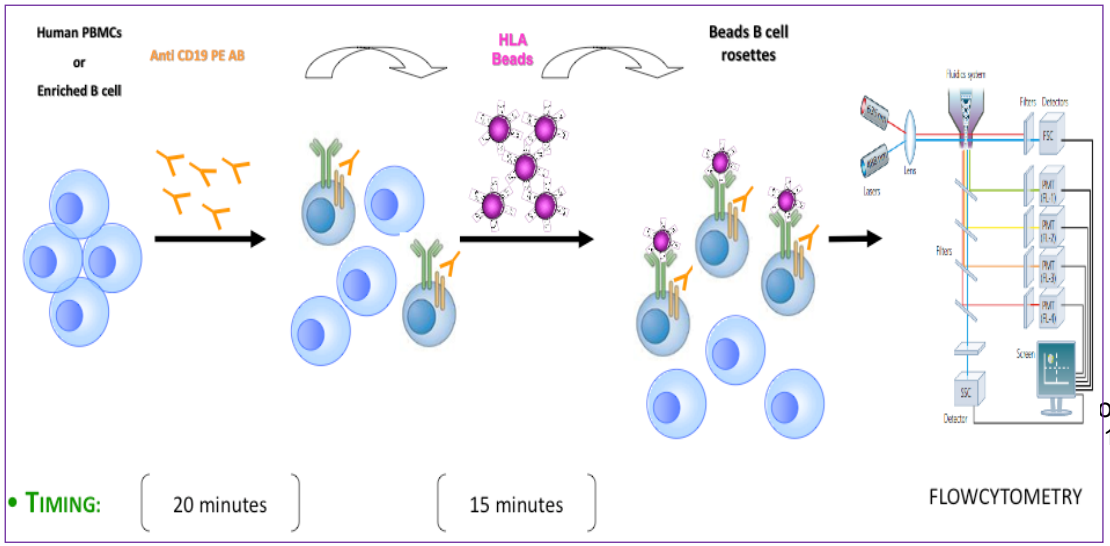
The transplant biopsy represents a sampling of the allograft and rejection is manifested as cellular infiltration, mostly of donor reactive T cells and B cells, classically detected through immunohistopathological staining and microscopic examination. An alternate approach that we have developed is to monitor the graft for infiltrating donor-reactive clonotype identified through AlloSEQ (17). This would entail isolation of total genomic DNA from the biopsies, performance of immunoSEQ analysis as above and monitoring for the presence and copy number of donor-reactive clones identified through the pre-transplant AlloSEQ, and then correlating with the severity of rejection monitored through immunohistopathological examinations.

We had proposed that transplant rejection can more easily be diagnosed through the sampling of the urine pellet or peripheral blood for clonal frequency and abundance of donor-reactive T cells pre-identified through AlloSEQ. This is to monitor rejection in a non-invasive manner.

We have already established the technology (17) and other investigators have used it to show that donor reactive clones are reduced or deleted in tolerant kidney transplants (21). Therefore, these proposed studies monitoring post-transplant graft rejection or its absence can be greatly leveraged to innovatively assess the relative efficacies of the drugs being compared.

Analysis of HLA and Donor-Specific B cells

Measurement of B cells in the peripheral blood with potential to make HLA antibodies against graft donor antigens might provide a way of analyzing antibody formation and shed light on the balance between production, inhibition and sequestration at any given time in the post-transplant period. Perry and coworkers have recently reported HLA antibody production in vitro by B cells from the bone marrow of sensitized kidney transplant recipients. T and B-cell ELISPOT have also been used to measure HLA specific B cell frequency against a given antigen. ELISPOT assay does not measure the frequency of cells that actually bind the antigen and only measures biological events such as cytokine release or production of immunoglobulin after differentiation in vitro. Furthermore, the estimated frequency is restricted to the cells that are able to be selectively stimulated by the antigen depending on read-out and thus may lead to an underestimation of the actual frequency of committed cells. Another approach based on the structural similarity between B cell receptor and immunoglobulin binding sites, it is postulated that HLA-specific B cells (HSB) should bind to HLA molecules with specificity comparable to that of the secreted immunoglobulin. Identification of HLA specific B cells by staining through binding of the B cell receptor using fluorescently labeled tetramers of identified HLA class I specificities has been described. A solid-phase assay utilizing



color-coded microspheres, each coated with a unique HLA antigen has become the main multiplex assay used for the detection of allo-specific antibodies against donor antigens. Its multiplexing power enables the analysis of the antibodies directed to all available synthetic HLA class I and class II alleles in one assay. Utilizing these single antigen HLA beads (SAB) Degauque et al. described a novel assay to identify HLA-specific B cells in transplant recipients (22). We have further developed this novel multiplex bead-based assay to identify HSB (schematically described in **Figures 5 and 6**), and propose here to study the circulating HSB in kidney transplant recipients with chronic AMR.

Figure 5: Assay Design

Proposed studies:

1. Frequency of Donor-specific B cells (DSB) by Novel HLA Bead Multiplex Assay: We will analyze the serial samples of peripheral blood mononuclear cells (PBMC) using our novel HLA-bead multiplex assay to identify and determine the changes in frequency of total B, HLA-specific B (HSB) and DSB cells in KT recipients with cAMR in each study arm.

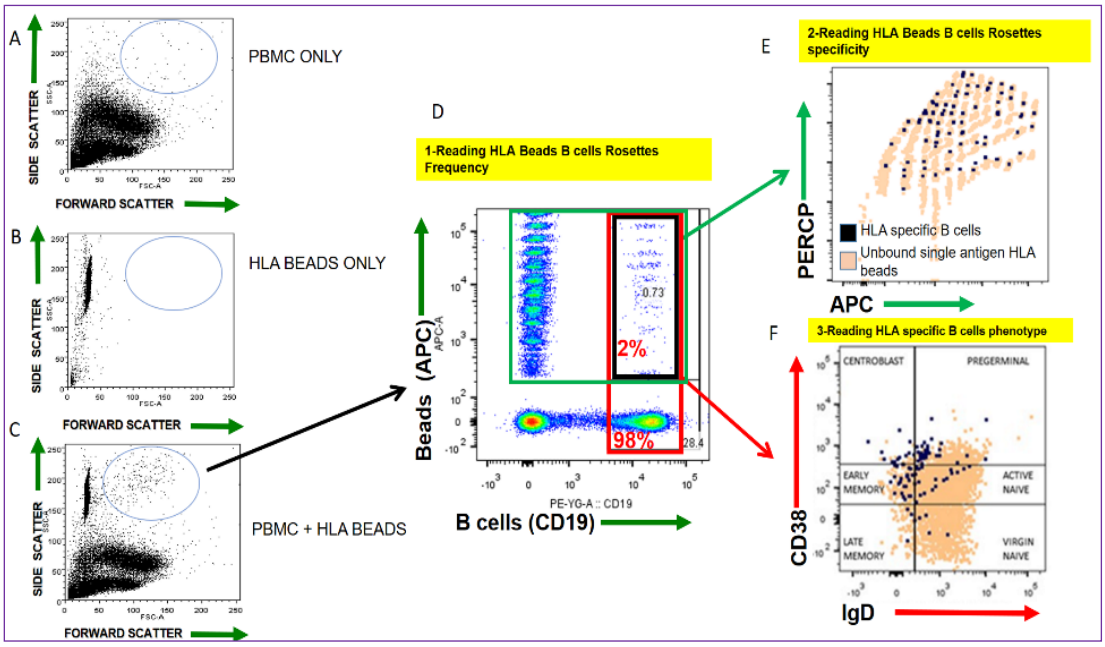


Figure 6: Analysis Strategy

2. Immunophenotyping of HLA-specific B cells and Donor-Specific B cells by Flow Cytometry: We will analyze serial samples of PBMC, before and at 3 and 12 months post-transplant, using multi-parameter flow-cytometry for immunophenotyping of (i) total B, HSB and DSB cells with surface expression of IgD, CD24, CD27 and CD38.

DATA AND SPECIMEN BANKING:

Samples collected will be banked at the Immune Monitoring Core (IMC) (under the leadership of Co-I Dr. James Mathew) of the Comprehensive Transplant Center (CTC) of Northwestern University. The blood immunophenotyping will be performed prospectively on fresh samples. The pre-transplant donor-specific MLR and flow-sorting of reactive T cells will also be performed with fresh samples. Cell pellets thus obtained (for AlloSEQ analysis) as well as post-transplant PBMC, urine sample pellets and biopsies will be frozen and sent blinded **(de-identified)** to Adaptive Biotechnologies, Seattle for DNA purification and ImmunoSEQ analyses. Leftover

samples of biopsies (paraffin-block after IHC staining), PBMC and urine pellets will be used for RNA isolation and gene expression profiling in a future study.

DATA AND SPECIMEN MANAGEMENT:

Statistical analysis:

The primary endpoints of the study are graft function and subpopulations of T cells, including regulatory T cells and B cell subpopulations as well as immune functions at 3 and 12 months post kidney transplant.

The secondary endpoints of the study include 1) incidence of acute rejection, graft loss, and death at 3 and 12 months post kidney transplant; 2) allograft immunohistopathology profiles at 3 and 12 months post kidney transplant.

We will run univariate and bivariate analyses for all variables prior to our main analyses. We will evaluate continuous variables using t-tests or F-tests and categorical variables using χ^2. We will examine data distributions and test all variables for linear relationships or non-linear relationships.

Descriptive statistics (means, standard deviation, frequency) and correlations (both Pearson and Spearman) among variables will be calculated for preliminary data assessment.

Necessary transformation and imputations will be carried out based on the raw data distribution.

As missing data are inevitable in a longitudinal study, we will determine whether missing data are MCAR (data are missing completely at random), MAR (data are missing at random), or NMAR (not missing at random). If missing data are MCAR or MAR, it is likely that the standard multivariate computations using PROC MI (multiple imputations) will not result in biased standard error estimates. However, if missing data are NMAR, we will use the "pattern mixture" approach to compute a weighted average of the parameters that are associated with the missing data to estimate what the data would have been.

For the primary objective, we hypothesize that subjects receiving LCP-Tacro/Envarsus® XR will exhibit significantly greater graft function and lower frequency / abundance of AlloSeq pre-identified donor-reactive T cell clones in the graft, peripheral blood and /or urine at 3 and 12 month post-transplant as compared to the standard of care tacrolimus group. A variety of covariance structures (first order regressive, compound symmetry, Toeplitz, variance components, unstructured) will be carefully examined and compared using the best fitting statistics, such as Akaike's Information (AIC) and Bayesian Information Criteria (BIC). These statistics are likelihood functions and can be compared across models. If, in spite of randomization, distributions of subjects' baseline characteristics are found to be different between the two experimental groups, these variables will be entered as covariates in the statistical analysis.

For the secondary endpoints, we will use logistic regression, Kaplan-Meier method, and Cox proportional hazards models to analyze time-to-event outcomes (acute rejection, graft failure, and death). For allograft immunohistopathology outcomes, we will first develop a comprehensive immune profile of each participant by longitudinal analysis of frequency of T and B cell subsets and function. We will examine how these correlate with immunohistopathology and alloantibody production using Pearson or Spearman correlations. We will compare correlation coefficients between the two experimental groups using Z -tests to determine the distinct effects of each treatment arm. We will also compare the trajectory changes in cell populations and antibody production over 3 and 12 months using both repeated measures ANOVA and random mixed effect models. All tests will be

two-sided, and an error rate of $\alpha < 0.05$ will be considered statistically significant. All data analyses will be performed using SAS 9.2 statistical software (SAS Inc., Cary, NC).

Sample size and power:
This proposal bases its sample size calculations on published data demonstrating increased peaks and troughs with conventional tacrolimus and absence of such peaks and troughs with LCP-Tacro/Envarsus® XR (1) and correlation with high doses and nephrotoxicity with conventional tacrolimus (4). Using a two-tailed α of 0.05, the anticipated effect sizes corresponding to each specific aim between the two treatment arms were calculated using a minimum of 80% statistical power and assumed 10% attrition at 12 months.

For the sample size of n= 25 in each group, the proposed study will have a power of 0.82 and 0.99 to detect effect size of 1.0 and 2.0 for graft function and immune activation (in terms of T cell clonal repertoire change), respectively, between the two treatment arms. Because this is a pilot study, we do not consider the statistical power of secondary outcomes; It is anticipated that a much larger sample size will be needed in order to achieve statistical power for the secondary endpoints in this proposed study.

Data Handling and Record Keeping
This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures. This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), for formal approval of the study conduct. Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following: 1) What protected health information (PHI) will be collected from subjects in this study 2) Who will have access to that information and why 3) Who will use or disclose that information 4) The rights of a research subject to revoke their authorization for use of their PHI. All subjects for this study will be provided with a printed consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, will be obtained before the subject is submitted to any study procedures. This consent form will be signed by the subject and the investigator-designated research professional obtaining the consent. The subject will be given a signed copy of the informed consent for their records. After study initiation, the electronic case report form (eCRF) will be the primary data collection instrument for the study. Only the study staff will have access to this password protected file. No subject identifiers will be included in the eCRF and electronic databases that are used to record clinical data on participants. All clinical data will be kept completely confidential. The PI, Dr. Lorenzo Gallon and the Co-Is Drs. Joseph Leventhal and Dr. Javeed Ansari or their designees will be responsible for the clinical data management.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS:

Safety:
Safety Endpoints: The primary safety endpoints will be assessed on the AEs and SAEs that are observed throughout the trial. For the purpose of this clinical trial, the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 3(CTCAE), dated June 14,

2010, will be used to grade all adverse events. The collection of AE's and SAE's is limited in this study to those Grade 3 or higher that are related to study mandated procedures.

Recording of Adverse Events
At each contact with the subject, the investigator/member of investigator's team will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded in the source document. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period that meet the criteria above will be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported according to IRB requirements.

Stopping Rules
Subjects developing post-transplant infections (i.e., UTI, CMV, HSV, EBV, HCV, HBV, HIV, PCP), that in the opinion of the investigator are detrimental to the subject and his/her participation in this research trial, will be stopped from participating in this study (even though these adverse events expected to be lower in the experimental arm of the study)**.** Patient Authorization: This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), (Northwestern University Institutional Review Board), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the investigator/sponsor before commencement of this study. The investigator will place a list of IRB members and their affiliate in the regulatory binder for the clinical trial. All subjects for this study will be provided with a printed consent form describing this study and providing sufficient information for subjects to make an informed decision.

WITHDRAWAL OF PARTICIPANTS:
Subjects may be withdrawn from the trial at any time for any reason, either by choice or due to medical indications. Subjects who are non-compliant with study guidelines, medications and clinic examinations will be withdrawn from the study. The IRB may also discontinue the study at any point. The subjects will be fully informed of these actions**.** If participants are withdrawn prematurely, they will still be followed through the follow-up time period of the study as per standard medical care. These data are important to collect to determine differences in this group compared to those who completed the study.

RISKS TO PARTICIPANTS:
The risks to the subjects are limited to the risks related to venipuncture and at least two passes of renal biopsies. Also, the routine risk of rejection with either of the drugs is expected; in fact, lower risk of nephrotoxicity is anticipated with LCP-Tacro/Envarsus® XR.

The National Cancer Institute Common Terminology Criteria for Adverse Events v4.03 (CTCAE), dated June 14, 2010 will be used to grade all adverse (either serious or non-serious) events. It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events. Information on all adverse events (signs, symptoms, abnormal diagnostic procedures, treatments) will be recorded in the appropriate adverse event module of the case report form (CRF). The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed to determine the final outcome.

Serious adverse events that are determined to be unexpected and at least possibly related to study participation will be submitted to the IRB within 5 business days of the investigator's knowledge of the event. Follow-up information will be submitted to the IRB as required. The IRB/Office for the Protection of Research Subjects at NU is under the direction of Nathalia Henry, MS, CIP, CHRC, (312) 503- 2578.

POTENTIAL BENEFITS TO PARTICIPANTS:
It is expected that participants in the experimental arm of LCP-Tacro/Envarsus® XR treatment will have lower nephrotoxicity, immune activation and associated improved clinical outcomes. These may be direct long-term benefits for participants, if they happen to be randomized into the study arm.

ECONOMIC BURDEN TO PARTICIPANTS:

None other than their own routine SOC expenses. A parking voucher will be given to the participants at their study visits.

VULNERABLE POPULATIONS:
None (Please see Inclusion and Exclusion Criteria)

SHARING OF RESULTS WITH PARTICIPANTS:
At the end of the study, at the PIs discretion, the participants may be informed of the incidental findings. This study will be registered at clinicaltrials.gov.

PRIOR APPROVALS:

All required approvals such as laboratory, radiation safety, or biosafety are already in place.

SETTING:
Renal transplantations will be performed by the co-I, Dr. Joseph Leventhal and his surgical colleagues at Northwestern Memorial Hospital. The clinical follow-up will be conducted as standard of care by the PI, Dr. Lorenzo Gallon and the transplant nephrologists including Dr. Javeed Ansari with the help of transplant nurse coordinator (TNC). The immune monitoring studies will be directed by Co-I, Dr. James Mathew with the help of Drs. Jie He and Xuemei Huang, two senior technologists in the Immune Monitoring Core of the CTC. Blinded **(de-identified)** samples will also be sent to Adaptive Biotechnologies, Seattle for ImmunoSEQ analyses. Leftover samples, if any, are expected to be discarded at the end of the study.

RESOURCES AVAILABLE:

Successful completion of the project requires the following: a) Team of dedicated investigators: Drs. Gallon and Ansari are transplant nephrologists and Dr. Leventhal is a transplant surgeon and they have dedicated their careers to translational research to refine the management of renal transplant recipients. They will actively recruit all patients and, assisted by an RN research coordinator at the CTC, perform all of the clinical assessments in this cohort. They will maintain the clinical database with help from the assistant coordinators. They will work closely with the co-I, Dr. James Mathew and his trained technologists in the Immune Monitoring Core of the CTC performing and interpreting the assays. Dr. Lihui Zhao will perform the statistical analysis of the immunophenotyping and Dr. Yashpal Kanwar will interpret the biopsies and IHC staining. Northwestern has formed a collaborative partnership with Adaptive Biotechnologies, Seattle for ImmunoSEQ analyses to identify TcR repertoire usage in donor-specific immune responses. A similar partnership has been established with Myriad-RBM for the analysis of kidney injury markers using their KidneyMAP panel, b) Ideal patient population: The Northwestern CTC transplant center provide the ideal infrastructure and performs a high number (>220) of living and deceased donor kidney transplant procedures/year to conduct this prospective trial and to analyze fresh patient samples. This readily supports recruiting 50 patients within one year enrollment period, even when considering the inclusion/exclusion criteria and other concurrent studies; c) Institutional support: Dr. Michael Abecassis, division head of the CTC will provide the full infrastructural support to conduct all clinical and laboratory portions of the study. In summary, the investigators, patient availability, and environment afford the highest chance of success in this proposal.

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