Northwestern University
Comprehensive Transplant Center
Feinberg School of Medicine

Liver Transplant Tolerance Enhanced By Sirolimus Therapy

Principal Investigator: Josh Levitsky, MD, MS
Northwestern University
Comprehensive Transplant Center
676 N. St. Clair Street, Suite 1900
Chicago, IL 60611
(312) 695-4413

Sub-Investigators: Joshua Miller, MD; James Mathew, PhD; Michael Abecassis, MD, MBA, Joseph Leventhal, MD, PhD, Daniel Ganger, MD, Sean Koppe, MD, Laura Kulik, MD, MS, Talia Baker, MD, John Fryer, MD, Juan Carlos Caicedo, MD, Anton Skaro, MD, PhD, Daniela Ladner, MD, MPH, Sam Rao, MD, Guang-Yu Yang, MD and Brittany Lapin, MPH

IRB Protocol Number: STU00072766

Date: 10-04-2017

Table of Contents

List of Abbreviations

AE	Adverse Event
ALK Phos	Alkaline Phosphatase
ALT	Alanine Aminotrasferase
AST	Aspartate Transaminase
AUROC	Area Under Receiver Operating Characteristic (Curve)
CBC	Complete Blood Count
CKD	Chronic Kidney Disease
CMP	Comprehensive Metabolic Panel
CNI	Calcineurin Inhibitor
CRF	Case Report Form
Cr	Creatinine
CTC	Comprehensive Transplant Center
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GGT	Gamma-Glutamyl Transpeptidase
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HbA1C	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immune Deficiency Virus

I/E	Inclusion/Exclusion
ICH	International Conference on Harmonization
IHC	Immunohistochemical
IL-2	Interleukin 2
IM	Immune Monitoring
IRB	Institutional Review Board
IS	Immunosuppression
ITN	Immune Tolerance Network
LFT	Liver Function Tests
LT	Liver Transplant
MDRD-4	Modification of Diet in Renal Disease-4 (version four)
MLR	Mixed Lymphocyte Reaction
NIAID	National Institute of Allergy and Infectious Disease
NIH	National Institutes of Health
NUIRB	Northwestern University Institutional Review Board
OLT	Orthotopic Liver Transplant
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
pLTQ	Post Liver Transplantation Quality of Life Instrument
PTLD	Post Transplant Lymphoproliferative Disorder
QOL	Quality of Life
RM-ANOVA	Repeated Measures o fAnalysis of Variance
SAE	Serious Adverse Event
SRL	Sirolimus

TAC	Tacrolimus
ULN	Upper Limit of Normal

Study Summary

Title	Liver Transplant Tolerance Enhanced By Sirolimus Therapy
Short Title	Sirolimus-Enhanced Tolerance
NU Protocol Number	STU00071766
Phase	Phase 4, pilot study
Methodology	Open label prospective study of Sirolimus withdrawal
Study Duration	18 months
Study Center(s)	Single-center
Objectives	Objectives: 1. To determine if tolerance can be achieved successfully in a reasonable percentage (>20%) of liver transplant (LT) recipients withdrawn from SRL monotherapy. 2. To investigate if blood/allograft metrics of immunoregulation correlate with successful SRL withdrawal in LT recipients.
Number of Subjects	25 Subjects meeting inclusion/exclusion criteria
Diagnosis and Main Inclusion Criteria	1) Adult LT recipients > 3 years post-LT and > 6 months on SRL monotherapy; 2) Primary living or deceased donor LT; 3) Consent to undergo full immunosuppression withdrawal (sirolimus [SRL] withdrawal)
Study Product, Dose, Route, Regimen	Sirolimus (patients on varying doses of daily oral tablet)
Statistical Methodology	The primary outcome will be the proportion of tolerant patients off SRL therapy with normal liver biochemistry and graft histology at 12 months. As a pilot study focused on acquiring preliminary data, there will be no control group maintaining SRL therapy or a control group withdrawing from different immunosuppressive therapy (i.e. calcineurin inhibitor [CNI] agents) as comparators. Immune assay changes with successful or failed withdrawal will also be compared with appropriate statistical tests.

This document is a protocol for a human research study. This study is to be conducted according to United States (US) and international standards of Good Clinical Practice (GCP); (Food and Drug Administration [FDA] Title 21, parts 312, 50, 56 and International Conference on Harmonization guidelines [ICH]), applicable government regulations and institutional research policies and procedures.

By signing below, I agree to conduct the research by US and International Standards of GCP FDA Title 21, parts 312, 50, 56 and ICH guidelines. Being the principal investigator (PI), I also agree to oversee that the subinvestigators and all research staff abide by the same standards and principles.

Name Date

Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 parts 312, 50, 56 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

The significance of this clinical trial lies in its potential to increase the success of immunosuppression (IS) therapy withdrawal in liver transplant (LT) recipients, thus decreasing the negative impact of IS on their long-term outcomes. Lifetime immunosuppression (IS) with standard agents, the calcineurin inhibitors (CNI) cyclosporine and tacrolimus (TAC), is currently required at clinically recommended doses and trough levels to prevent allograft rejection. However, this occurs at the significant expense of long-term CNI toxicity, i.e. chronic kidney disease (CKD), hypertension, hyperlipidemia, diabetes, infections and malignancy (1, 2). With improvements in early graft and patient survival, long term adverse IS effects have become increasingly important in this rapidly expanding patient population. The strategies to reduce long term CNI toxicity include dose minimization that still leaves patients on CNI therapy, conversion to non-CNI therapy, or even complete IS withdrawal. The second approach, conversion to non-CNI IS therapy, is attractive in the potential to stabilize or improve renal function and other CNI toxicities. One such non-nephrotoxic IS agent, the mammalian target of rapamycin inhibitor (mTOR-I) SRL, has a different mechanism of IS action and studies have shown that CNI to SRL conversion can stabilize renal dysfunction with a low risk of rejection (3, 4). Yet even with these possible benefits, patients on SRL are still subject to lifetime IS therapy with side effects and costs, highlighting the need to investigate the strategies that promote full IS withdrawal without rejection (3rd approach), also known as 'operational tolerance'.

Therefore, specific to this proposal and supported by our preliminary data, an additional advantage of mTOR-I therapy lies in its potential to promote such a clinical immunoregulatory state that could facilitate IS minimization and complete withdrawal. As the most immunoregulatory solid organ transplanted, the liver houses numerous extramedullary hematopoietic and non-immunogenic cells and secretes several immunoregulatory proteins (5). As a result, the percentage of LT recipients able to undergo IS withdrawal is the highest of all solid organ transplant recipients, although still only ~ 20% successful to date and primarily only performed in CNI-treated patients in withdrawal studies (6). This clinically unacceptable percentage is likely due to known CNI mechanisms inhibiting immunoregulation and the lack of available, well-defined immune monitoring to detect immunoregulation or unresponsive states. A key difference between mTOR-I and CNI is their effect on regulatory T cells (Tregs) and regulatory 'immature' dendritic cells (DCregs) important in the suppression of auto- and allo-immune responses. As an inhibitor of IL-2 signaling after T cell activation, SRL blocks proliferation of alloreactive T cells but promotes Tregs ($CD4^+CD25^{high}FOXP3^+$), regulatory cytokines (i.e. TGF-β1), and tolerogenic dendritic cells (DCreg) (7, 8). In contrast, CNIs inhibit interleukin-2 (IL-2) transcription and thus negatively affect Treg generation (9-11). Importantly and most relevant to this proposal, we have recently demonstrated systemic (peripheral blood mononuclear cell [PBMC], bone marrow, allograft) Treg/DCreg increases and enhanced tolerogenic proteogenomic markers in LT recipients converted from CNI to SRL (12, 13), as well as augmented allo-specific Treg function by SRL vs. CNIs *in vitro* (14). These data provide the clinical and mechanistic background that support SRL facilitating operational tolerance in this pilot study.

The identification of biomarkers of tolerance is as important as the chosen IS regimen. Various reports demonstrate a high percentage of Tregs, DCregs, γδ Tcells (Vδ1/Vδ2 ratio), and specific gene signatures in tolerant LT recipients (6, 15, 16). We have utilized a number of *in vitro* assays testing donor-specific

Treg inhibition/recruitment (Treg MLR) and regulation of cytotoxic T cells (micro-cell mediated lympholysis, m-CML) induced by IS therapies and in clinical tolerance trials (14, 17-20). Also, we have utilized peripheral blood/allograft immunophenotyping for regulatory cell populations and immunoregulatory proteogenomic assays to look for signatures of tolerance and CKD in LT recipients (13, 21). Yet the presence of Treg populations and differential gene array snapshots in 'already-withdrawn' patients or patients currently on IS therapy, without a clear understanding of their utility in monitoring during withdrawal, only provides circumstantial evidence for their role in tolerance. An array of assays performed throughout the tapering and withdrawal period, as described herein, would provide a more accurate measure of the effects of SRL on Treg function and immunoregulatory gene/protein expression, supporting their use as predictors of tolerance in future protocols.

With the above background and rationale, we expect to develop two strategies to promote the ability to achieve and monitor LT tolerance. The first is initial CNI to SRL conversion to promote systemic immunoregulation (already performed in our preliminary work (13)), followed by SRL minimization and withdrawal (this Institutional Review Board [IRB] submission). This is to be compared to the established literature success rate (≈20%) of CNI withdrawal, as we do not feel it is clinically acceptable to include this as a control group (6). *We hypothesize that the clinical use of SRL promotes immunoregulatory pathways and provides higher IS withdrawal success compared to CNI therapy*. The second strategy aims to identify immunologic and proteogenomic assessments of immunoregulation, potentially enhanced by SRL conversion and predicting/correlating with successful IS withdrawal.

In summary, even though this is a small clinical/translational pilot study, it has a potential for significance in the field of transplant medicine. If successful, it could serve as a model approach for further studies and national grant funding in LT tolerance. Specifically, the Immune Tolerance Network (National Institutes of Health/National Institute of Allergy and Infectious Disease [NIH/NIAID]) requests these pilot data from our center prior to conducting a large multicenter controlled study of SRL (or other mTOR-I) withdrawal in LT recipients. The downstream impact could be far-reaching, not only from a clinical standpoint in improving patient outcomes, but also lead to amplified research in mechanisms of mTOR-I therapy, other tolerogenic approaches and biomarkers of tolerance. The immunoregulatory signatures determined preliminarily in this study could eventually, if validated, facilitate 'point-of-service' tests in clinical settings, either routinely or during modifications of therapy. Knowledge of such specific clinical and immunological characteristics and using an individualized approach would allow transplant clinicians to more accurately define and select appropriate candidates for withdrawal. This would satisfy our long term goal of developing a refined approach to more successfully withdraw IS therapy and improve outcomes for LT recipients.

1.2 Preclinical Data

Preliminary Data: We demonstrated that liver transplant (LT) recipients on SRL monotherapy had higher percentages of peripheral blood mononuclear cell (PBMC) immunophenotypic Tregs (CD4+CD25Hi+Foxp3+ cells) compared to recipients on CNIs (12).This led to a recently published CNI to SRL conversion trial demonstrating increased percentages of PBMC/bone marrow Tregs and PBMC DCregs (ILT3/4+) in LT recipients converted from tacrolimus to SRL monotherapy(13). Liver biopsy immunohistochemistry FOXP3:CD3 and CD4:CD8 ratios were significantly higher after conversion. Patient sera on TAC but not SRL suppressed Treg generation in mixed lymphocyte reactions. Finally, consistent with prior reports of genomic expression patterns and cellular assays as signatures of tolerance (16), we detected the expression of 289 novel genes and 22 proteins, several important in immunoregulatory pathways, after SRL conversion. In a separate in vitro study, we also demonstrated robust allospecific regulatory effects of SRL in Treg mixed lymphocyte reactions (14). Our preliminary data, in conjunction with other clinical and laboratory reports, provide the mechanistic rationale for the

concept that SRL may facilitate IS withdrawal in LT and that such assays should be tested in parallel as biomarkers of tolerance. We hypothesize that SRL will promote regulatory gene/protein expression patterns in the peripheral blood AND in the allograft, which can then be utilized as predictive assays in tolerance trials. To robustly test this hypothesis, we will collect serial blood and allograft samples before/after withdrawal and at the time of biopsy for possible rejection, all to be tested as markers of immunoregulation. This will consist of assays similar to those performed in our recent work (12, 13, 21).

1.3 Clinical Data to Date

We and others have shown the safety of CNI to SRL conversion (3, 4, 12, 13), but there are no current studies testing full IS withdrawal in patients on SRL monotherapy. There are numerous studies of CNI withdrawal that have shown together an approximate 20% success rate (6). Fortunately, the risk of graft failure due to rejection in all of these prior withdrawal studies was extremely low (<1%) (6), making withdrawal a reasonably safe option. However, even so, 20% success is not considered clinically high enough to be acceptable as a standard of care approach and few centers, if any, are withdrawal patients routinely from IS therapy because of this low rate, except for in the setting of aggressive malignancy, infection or post-transplant lymphoproliferative disorder (PTLD). Thus, our goal is that SRL will be associated with higher than 20% success, allowing further study of this approach to make IS withdrawal clinically more feasible and acceptable to clinicians.

1.4 Dose Rationale and Risk/Benefits

All our LT patients are on different doses of SRL therapy currently (on average 1-2 mg daily) and will be tapered off of SRL according to the study procedures. There is no new drug therapy introduced in this study. It is hypothesized that 50% (vs. 80% historical CNI withdrawal) will develop reversible rejection during either minimization or withdrawal of SRL, requiring reinstitution of immunosuppressive therapy (i.e. failure of withdrawal). Fortunately, previous studies involving withdrawal of IS in LT recipients (mainly from CNI therapy) have shown that patients who fail to withdraw and develop rejection have a minimal (<1%) risk of graft failure when IS reinstituted (6). Only one patient in the major withdrawal studies developed chronic rejection and required retransplantation. Thus it is, in the vast majority, reversible. This may not be the case with SRL therapy although none of our previously SRL converted patients developed rejection or lost their grafts. Therefore, we feel the risk of withdrawal, while not insignificant, is fairly low in comparison to the high potential for benefit of being completely withdrawn from IS therapy altogether, satisfying the concept of clinical equipoise. All of these risks and benefits will be discussed with the patient at the time of informed consent. For IS withdrawn patients in our study, we expect at minimum stability or improvement in all of the secondary outcomes related to chronic IS therapy. For liver biopsies on all patients, the risk includes the following: bleeding, infection, perforation of an organ surrounding the liver (all < 1%). The patient will undergo a separate informed consent (for the procedure and is different than the actual study consent) prior to all liver biopsies. We feel that liver biopsy is necessary so that the patient will not proceed into the trial if any histological graft dysfunction or rejection is detected on biopsy. While liver biopsy is invasive and has associated risks, they are outweighed by the benefit of knowledge of liver histology prior to the intervention and follow-up. Decisions on whether to undergo the intervention can therefore be more accurately determined and provide more safety than risk to the patient. All of these risks will be clearly discussed with the patient during the study process and documented by appropriate consents.

2 Study Objectives

Primary Objective: The goal is to determine if operational tolerance (normal graft function without IS therapy x 12 months) can be achieved successfully in LT recipients withdrawn from SRL.

Secondary Objectives

1. To investigate if cellular, allograft and proteogenomic metrics of immune regulation are predictive of and persist following successful SRL withdrawal in LT recipients.

2. To analyze any initial clinical benefit of minimization and withdrawal of SRL therapy.

3 Study Design

3.1 General Design

Study Overview

The study proposed is a prospective trial of controlled SRL monotherapy minimization and withdrawal in up to 25 stable non-immune non-viremic LT recipients. Given the sample size calculations (see Statistical section), we plan to enroll up to 25 SRL monotherapy patients for this study (Figure 1). All patients will be consented to undergo laboratory evaluation as well as an enrollment liver biopsy and blood tests. If the patient meets inclusion/exclusion (I/E) criteria (see below), SRL will be minimized over 6 months until once a week dosing is achieved. Repeat clinical and immunological blood tests as above will be performed, and if no biochemical signs of rejection, SRL will be discontinued with blood tests and liver biopsy 12 months later for similar biochemical, histological and immunological measures. At any concern for rejection, liver biopsy and assays for equivalent biochemical, histological and immunological measures will be performed and if rejection is diagnosed on biopsy, a second attempt at withdrawal **will not** be performed. Patients will be monitored as standard of care with clinic visits every 6 months and laboratory tests every 2 weeks. The total study length will be 18 months: 6 month minimization phase and 1 year follow-up after withdrawal success/failure. The primary outcome will be the proportion of tolerant patients off SRL therapy with normal liver biochemistry and graft histology at 18 months. Secondary outcomes will include the incidence, severity and reversibility of rejection, patient/graft survival, resolution of SRL and other non-specific IS effects, and the assessment of clinical/immunological biomarkers of tolerance. All continuous/categorical clinical variables will be compared with the appropriate statistical analyses.The goal is that the primary and secondary aims will provide valuable preliminary data to further elucidate the mechanisms of mTOR-I immunoregulation and for determining the initial clinical success/feasibility of the mTOR-I approach (vs. historical 20% CNI withdrawal success seen in studies from the Immune Tolerance Network [ITN] and other groups). This is all so as to guide a submission to the Immune Tolerance Network for larger, more adequately powered prospective trials comparing SRL vs. CNI withdrawal and accompanying biomarker predictors of tolerance. If this pilot study fails to show a correlation between our biomarkers and the success/failure of SRL withdrawal, or is associated with an unacceptable low (e.g. <20%) rate of operational tolerance, then this would avoid the necessity for such large, expensive trials and support the continued development of alternative approaches to tolerance.

Consent and Initial Phase of Enrollment (see Study Protocol Figure- Appendix A)

These liver transplant candidates (up to 25 SRL) will be approached for consideration and informed consent into the study. The consent form will include discussion of the risks/benefits of their current therapy (SRL) and the planned minimization/withdrawal. Specifically, the risks of minimization/withdrawal (i.e. developing acute or chronic rejection, alloimmune hepatitis during any portion of the study), although unlikely, will be a major emphasis of the consent process. The consent form will also include the strict requirement for patients to follow all instructions from the PI in regard to the close laboratory follow-up occurring throughout the trial, to diagnose any episode of rejection or concern as early as possible to be able to respond appropriately. All of the study procedures will be discussed with the patient during clinic visits. They will be informed that neither participation nor refusal will influence their clinical care. All laboratory tests or costs related to their care in the study, with the exception of non-standard of care items (i.e. liver biopsies, blood assays), will be the responsibility of the

patient and/or his/her insurance company. They will be asked questions afterwards to verify comprehension and then sign the consent form documenting their agreement to participate. A signed copy of the consent form will be given to them. Participation is completely voluntary and they may discontinue participation the study at any time without affecting their care or participation in any other study. No financial compensation will be given.

Screening Evaluation at Enrollment (see Study Protocol Figure)

After long term (> 3 years post-LT, > 3 months on SRL monotherapy) on SRL monotherapy, inclusion/exclusion criteria will be reviewed and if appropriate, consent as above will be obtained. If the patient agrees and signs consent, baseline standard of care screening laboratories complete blood count, comprehensive metabolic panel, sirolimus (CBC, CMP, SRL trough level, fasting lipid profile, hemoglobin A1C [HbA1C], urine protein/creatinine ratio) and non-standard of care biomarker assays (blood immunophenotyping, proteogenomics) and liver biopsy (histology and graft immunohistochemistry) will be performed as dictated by the protocol. In addition, the liver biopsy will be used to determine stability in graft function (i.e. no evidence of rejection or immune-mediated hepatitis) before considering minimization/withdrawal. SRL minimization/withdrawal will only be performed if clinically, biochemically and histologically [by biopsy; liver transplant pathology read (Yang, Rao)] stable. Throughout the entire study, liver function tests will be monitored every 2 weeks (monthly is standard of care, so the interim non-standard of care 2 week blood tests will be covered by the study funds). Patients entering the minimization/withdrawal phases will be reduced by a total dose of 50% of their baseline dose every month until patients are on 0.5 mg SRL daily. At this point, every other day dosing will begin x 1 month. If no LFT abnormalities, twice weekly dosing x 1 month, then once a week dosing x 1 month. Prior to complete discontinuation, repeat clinical (screening labs above) and blood biomarker assays (blood immunophenotyping, proteogenomics) will be performed. Liver biopsy will not be performed at this juncture unless there are biochemical signs of liver injury. If complete withdrawal is deemed safe (no evidence of biochemical abnormalities) patients will be withdrawn completely (i.e. the once/week SRL dose discontinued) and followed off IS therapy for one year. In this time period, liver function tests will be monitored every 2 weeks, as usual, and repeat clinical (screening labs above), liver biopsy and blood (blood immunophenotyping, proteogenomics) assays performed at the end of this year or at any concern for rejection.

Blood and Tissue Samples

Standard of care laboratories (CBC, CMP) will be performed every month on all patients, and only LFTs performed in the interim 2 weeks between each month (non-standard of care; covered by the study funds) until complete withdrawal. 3 green top 5 mL and 3 red top 5 mL tubes of blood for biomarker assays will be taken on all patients prior to study enrollment, prior to complete withdrawal, and one year after withdrawal or at the time of suspected rejection. Liver biopsy (one 3 cm biopsy- 2 cm for histology and 1 cm for genomic assays) will be performed at baseline (pre-minimization) and 1 year post withdrawal. A separate clinical informed consent will be obtained each time a liver biopsy is performed. If the patient develops elevation in liver transaminases requiring a liver biopsy at any stage of the study protocol, blood (all for immune monitoring (IM) assays) will be requested from the patient at the time of the biopsy. If rejection occurs, the patient will be followed in the study until completion but not be further withdrawn from SRL or have a liver biopsy at the end of the study

Follow-up

Laboratory follow-up is described above- every 2 weeks throughout the trial until complete withdrawal. Any biochemical (or clinical) signs or significant liver function test abnormalities will be acted on immediately either by liver biopsy or a pause in IS withdrawal per investigator discretion. All patients will be seen in the clinic every 3 months to assess for any new signs or symptoms or resolution of drug side effects in the minimization or withdrawal arm. Quality of life questionnaires; Post Liver Transplant

Quality of Life Instrument (pLTQ) and the Promis-29 profile v1.0 will be administered at the study onset and after successful vs. unsuccessful withdrawal (end of study)

Outcome Measures

The primary outcome will be the proportion of patients off SRL therapy with normal liver biochemistry and graft histology at 12 months (i.e. tolerant). Thus, the incidence of graft dysfunction (acute rejection, immune mediated or autoimmune hepatitis, chronic rejection) or non-tolerance will be assessed in this SRL withdrawal group and compared to the historical CNI group (20% tolerant; 80% failure) as the primary endpoint. This rate will be a composite of the cumulative number of biopsy-proven graft dysfunctions requiring conversion back to SRL or additional IS therapy or discontinuation of minimization/withdrawal that occur during the course of the study. Major secondary measures compared will be the predictive capacity of the blood and graft biomarker assays (immunophenotyping, genomic/proteomics) before and after minimization/withdrawal in the success vs. failure groups. Clinical secondary outcomes will be compared: the number of infectious complications, liver biopsy complications, cardiovascular outcomes (i.e. blood pressure, diabetes control, lipid levels), renal function, hematopoietic parameters, gastrointestinal effects, or other side effects of SRL that may or may not improve or develop with minimization/withdrawal. These will all be documented by a study database during patient visits, electronic charts and/or by phone communication. Finally, quality of life (Post Liver Transplant Quality of Life Instrument (pLTQ) and the Promis-29 profile v1.0) will be analyzed at the end of the study to determine the effect of IS minimization/withdrawal on other health benefits.

3.2 Primary Study Endpoints

The primary outcome will be the proportion of tolerant patients off SRL therapy with normal liver biochemistry and graft histology at 12 months.

3.3 Secondary Study Endpoints

Secondary outcomes will include the incidence, severity and reversibility of rejection, patient/graft survival, resolution of SRL and other non-specific IS effects, and the assessment of clinical/immunological biomarkers of tolerance.

3.4 Primary Safety Endpoints

Safety of the study will be monitored closely and determined by the the incidence, timing, severity and reversibility of rejection with withdrawal, graft function (biochemical and histological) throughout and at the end of the study, resumption of other IS therapies due to rejection and their toxicities (SRL,CNIs, prednisone, mycophenolic acid, other), complications from phlebotomies and liver biopsies, and patient/graft survival (patient/graft loss very unlikely to occur based on prior studies)

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1) Adult male and female recipients of all races, ≥ 18-75 years of age
2) Patients who underwent primary living or deceased donor liver transplantation ≥ 3 years (previous to screening) and on ≥ 3 months of stable SRL monotherapy
3) Recipient of single organ transplant only
4) Liver transplant for non-immune, non-viral (no hepatitis B or hepatitis C virus unless currently non-viremic) causes
5) Ability to provide informed consent and to comply with the study protocol of IS withdrawal.

4.2 Exclusion Criteria

1) Inability or unwillingness to provide informed consent
2) Acute cellular rejection within 12 months prior to enrollment
3) Viral (viremic hepatitis B virus [HBV] or hepatitis C virus [HCV]) or immune-mediated liver disease (Autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis) history
4) Abnormal liver function tests: Direct bilirubin ≥ 1 mg/dL; ([ALT, AST, GGT] or alkaline phosphatase [AlkPhos] ≥ 2x [ULN]); 5) Abnormal graft histology at enrollment: a) ≥ Grade 2 inflammation or stage 2 fibrosis; b) Acute or Chronic Rejection; c) De-novo Autoimmune Hepatitis; d) inflammation of >50% of portal tracts; e) Other pathology not-specified but deemed high risk per the PI and pathologist; 6) Ongoing or recurrent substance abuse
7) Retransplantation or combined liver-other organ
8) Human Immunodeficiency Virus(HIV) co-infection
9) Glomerular Filtration Rate (GFR)<30 ml/min by estimated glomerular filtration rate ([eGFR]-[MDRD-4])

4.3 Subject Recruitment and Screening

Recruitment and Informed Consent

Nonimmune, nonviremic liver transplant patients on SRL therapy meeting I/E criteria (except for liver biopsy which will be performed only if all other I/E criteria met) will be approached consideration and informed consent into the study. The consent form will include discussion of the risks/benefits of their current therapy (SRL) and the planned minimization/withdrawal. Specifically, the risks of minimization/withdrawal (i.e. developing acute or chronic rejection, alloimmune hepatitis during any portion of the study) and procedural risks will be a major emphasis of the consent process. The consent form will also include the strict requirement for patients to follow all instructions from the PI in regard to the close laboratory follow-up occurring throughout the trial, to diagnose any episode of rejection or concern (elevation of liver transaminases) as early as possible to be able to respond appropriately. All of the study procedures will be discussed with the patient during clinic visits. They will be informed that neither participation nor refusal will influence their clinical care. All laboratory tests or costs related to their care in the study, with the exception of non-standard of care items (i.e. liver biopsy, IM assays), will be the responsibility of the patient and/or his/her insurance company. They will be asked a few questions afterwards to verify comprehension and then sign the consent form documenting their agreement to participate. A signed copy of the consent form will be given to them. Participation is completely voluntary and they may discontinue participation in either study at any time without affecting their care.

Screening Evaluation at the time of Minimization/Withdrawal

If the patient agrees and signs consent, history and physical, QOL (quality of life) survey, and screening laboratories (CBC, CMP, Trough IS level, HBA1C, Lipid profile, Urine prot/cr ratio) will be performed. Following this, single pass liver biopsy (histology and graft immunohistochemical [IHC] assays) and blood assays will be performed as part of the immunological monitoring protocol. In addition, the liver biopsy will be used to determine stability in graft function (i.e. no evidence of rejection or immune-mediated hepatitis) before considering minimization. SRL minimization/withdrawal will only be performed if clinically, biochemically and histologically (by biopsy) stable, following the I/E criteria. Patients entering the minimization/withdrawal phases will be reduced every month by 50% of total dose until they reach 0.5 mg daily for one month. They will then be reduced further by to 0.5 mg every other day, then twice weekly, then once weekly (all separated by one month at a time) dosing. This should take approximately 6 months to complete the minimization phase as most patients are currently on 1-3 mg of SRL daily. Throughout the study time period, liver function tests will be monitored every 2 weeks. For

any patient throughout the study developing liver dysfunction (direct bilirubin >1 mg/dL; ALT, AST, or AlkPhos > 2xULN), this will be confirmed on a repeat blood test within 1-2 weeks and if persistent, liver biopsy will be performed. Immediately before complete withdrawal, i.e. repeat clinical (screening labs) and blood assays will be performed. If complete withdrawal is deemed safe (no evidence of biochemical abnormalities, i.e. direct bilirubin < 1 mg/dL; ALT, AST, or AlkPhos < 2xULN), patients will be withdrawn completely (i.e. the once/week SRL dose discontinued) and followed off SRL therapy for one year. In this time period, liver function tests will be monitored every 2 weeks. At 12 months following withdrawal, the same history and physical, QOL survey, screening labs and blood IM assays will be performed as in the study initiation, as well as the liver biopsy- all for the primary endpoint. If rejection or immune-mediated hepatitis is seen on this post-withdrawal biopsy or at any time during the study period, the patient will be reinitiated on IS therapy appropriately per the PI and not have another withdrawal attempt. All patients will be followed until 1 year post-attempted withdrawal, even if unsuccessful, although patients developing rejection or resumption of IS prior to this period will not have a final liver biopsy performed (i.e. only those presumed tolerant or who did not have rejection).

Blood and Tissue Samples
Standard of care laboratories complete blood count, liver function tests (CBC, LFTs) will be performed every 2 weeks on all patients. Please see Figure 1 above for sample collections. A separate clinical informed consent will be obtained each time a liver biopsy is performed. If the patient develops elevation in liver transaminases requiring a liver biopsy at any stage of the minimization/withdrawal phase, blood assays will be requested from the patient at the time of the biopsy. These immunological data in rejecting patients will be important to compare to patients who successfully are minimized and withdrawn.

Follow-up
Laboratory follow-up (CBC, CMP) is described above- every 2 weeks throughout the trial. Any biochemical (or clinical) signs or significant liver function test abnormalities will be acted on immediately either by liver biopsy or resumption of the recent IS dose. All patients will be seen in the clinic every 3 months to assess for any new signs or symptoms or resolution of drug side effects in the minimization or withdrawal arm. The Post Liver Transplantation Quality of Life and Promis-29 quality of life surveys and IS cost tally will be performed prior to the study onset and after successful vs. unsuccessful withdrawal (end of study).

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects may be withdrawn from the trial at any time for any reason, either by personal 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 NU IRB may also discontinue the study at any point. The subjects will be fully informed of these actions.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If patients are withdrawn prematurely, they will still be followed through the follow-up time period of the study and beyond as per standard medical care. These data are important to collect to determine differences in this group compared to those who completed the study. This will include patient and graft survival data, as well as laboratory values and immunosuppressive therapy. If the subject is lost to follow-up, we will make every attempt to contact the patient (i.e. phone calls, certified letters, attempts at contacting next-of-kin, etc).

5 Subject Compliance Monitoring

5.1 Subject Compliance Monitoring

The investigator and research coordinator will have full contact with the patient throughout the course of the study. Subjects will be seen in the clinic at baseline and every three months through the study. Laboratory tests will be monitored by the investigator and research coordinator, and lapses in compliance with laboratory tests will be determined by monitoring a schedule of events and follow-up for each patient. In addition, the patient will be called on a monthly basis prior to each tapering of SRL therapy to document compliance with the dosing and for any new symptoms. Persistent non-compliance will be an indication to remove the patient from the study, per investigator discretion.

5.2 Prior and Concomitant Therapy

All concomitant medicines will be collected at each study visit (ie; every 3 months). There are no restrictions on concomitant medicines/therapies except for additional immunosuppression (IS) therapy or other therapies deemed unsafe per the investigator.

6 Study Procedures

6.1 **Visit 1: Screening/Pre-Withdrawal:** (occurs at a Standard of Care Visit): If the patient agrees after reading through the consent and discussing the study with the study physician and/or research coordinator/nurse they sign the consent, then a history and physical will be performed. The Post Liver Transplantation Quality of Life and Promis-29 quality of life surveys will be administered and, laboratory tests (CBC, CMP, Trough SRL level, HBa1C, Lipid profile, Urine prot/cr ratio, and tolerance/immunophenotyping/genomic/proteomic assays) will be performed.

6.2 **Visit 2: Liver Biopsy (+2 weeks of Screening/Pre-Withdrawal Visit)**: Single pass liver biopsy (histology and graft immunohistochemistry assays) and blood immune/biomarker (tolerance/immunophenotyping/genomic/proteomic) assays will be performed as part of the immunological monitoring protocol.

6.3 **Monitoring during Minimization (No visits):** SRL minimization will only be performed if clinically, biochemically and histologically (by biopsy) stable, following the I/E criteria. Patients entering the minimization phases will be reduced every month by 50% of total dose until they reach 0.5 mg daily for one month. They will then be reduced further by to 0.5 mg every other day, then twice weekly, then once weekly (all separated by one month at a time) dosing. This should take approximately 6 months to complete the minimization phase as most patients are currently on 1-3 mg of SRL daily. Throughout the study time period, liver function tests will be monitored every 2 weeks. For any patient throughout the study developing liver dysfunction (direct bilirubin >1 mg/dL; ALT, AST, or AlkPhos > 2xULN), this will be confirmed on a repeat blood test within 1-2 weeks and if persistent, liver biopsy will be performed.

6.4 **Visit 3: End of Minimization and Pre-Withdrawal (6 month clinic visi**t): Immediately before complete withdrawal, i.e. repeat clinical (screening labs as SOC, except SRL level not needed) and blood (tolerance/immunophenotyping/genomic/proteomic- research) assays will be performed. If

complete withdrawal is deemed safe (no evidence of biochemical abnormalities, i.e. direct bilirubin < 1 mg/dL; ALT, AST, or AlkPhos < 2xULN), patients will be withdrawn completely (i.e. the once/week SRL dose discontinued) and followed off SRL therapy for one year. In this time period, liver function tests will be monitored every 2 weeks as per 6.3 above. A history and physical will be performed as SOC (every six months visits are SOC at our transplant center).

6.5 ***Visit 4: Post-Withdrawal (12 month clinic visit- SOC):*** history and physical, laboratories (CBC, CMP) will be performed. In this time period, liver function tests will be monitored every 2 weeks as per 6.3 above.

6.6 ***Visit 5: Post-Withdrawal (18 month clinic visit- study end):*** At 12 months following withdrawal, the same history and physical, the Post Liver Transplantation Quality of Life and Promis-29 quality of life surveys, labs (CBC, CMP, HBa1C, Lipid profile, Urine prot/cr ratio- SOC; and tolerance/immunophenotyping/genomic/proteomic assays- research) will be performed as in the study initiation, as well as the research liver biopsy- all for the primary endpoint. If rejection or immune-mediated hepatitis is seen on this post-withdrawal biopsy or at any time during the study period, the patient will be reinitiated on IS therapy appropriately per the PI and not have another withdrawal attempt. All patients will be followed until 1 year post-attempted withdrawal, even if unsuccessful, although patients developing rejection or resumption of IS prior to this period will not have a final liver biopsy performed (i.e. only those presumed tolerant or who did not have rejection).

7 Statistical Plan

7.1 Sample Size Determination

We do not plan to utilize a control/non-withdrawn or CNI comparator group as 1) this is an initial pilot mechanistic study; 2) patients can serve as their own control (rejection vs. success) for this initial effort; 3) our prior studies have shown that statistically significant changes in the blood/graft biomarkers can be detected with just 20 patients. These results will be critical in guiding power calculations for larger, prospective, controlled studies testing the specific biomarker predictors that are identified herein and whether SRL vs. CNI withdrawal is associated with a higher % of tolerance. In addition, our sample size is limited by our available funds and the numbers of patients on SRL monotherapy in our center (estimated around 40)

7.2 Statistical Methods

We have performed within subject comparisons and measurements on PBMC and biopsy using appropriate paired analysis (paired t-test or equivalent nonparametric test (Wilcoxon Signed Rank test) (12, 13). Repeated measures of analysis of variance (RM-ANOVA) were used to test the trajectory variations over time. Two-sided F-test statistics were applied using α=0.05. For proteogenomic bioinformatics analysis, we have reported for each gene/protein comparison the T-test p-value, its non-parametric analog (Kruskal Wallis test), the fold change, and for multi-analyte signatures, the AUROC curve (C-index) and the q-values by member genes/proteins. We were then able to calculate the predictive accuracy, sensitivity and specificity of each signature. For clinical endpoints, categorical and continuous variables will be analyzed with appropriate tests (Fisher's exact, T-test). $P<0.05$ will be considered significant

7.3 Subject Population(s) for Analysis

All subjects (successful vs. failed, withdrawn from the study vs. maintained) will be followed and analyzed at study end. We will also document the number of patients screened who did not meet I/E criteria at the study initiation.

8 Safety and Adverse Events

8.1 Definitions

Adverse Event

By definition an ***adverse event*** (AE) is defined as: any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Adverse events are classified as serious or non-serious.

Serious Adverse Event

. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Events that are expected, anticipated, related to the standard of care treatment or known adverse events/serious adverse events from standard of care medications or pre-existing conditions will not be reported.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious will be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

For the purposes of this study, Adverse events and Serious Adverse events will be defined as those that are related to the procedures performed specifically for the study which are:

liver biopsy (regardless if performed percutaneously, transjugularly or open) and phlebotomy which takes place only to draw study-related blood. The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up the last visit made for the study.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. The pre-existing conditions will be recorded prior to the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator/designee must seek information on adverse events by specific questioning and, as appropriate, by examination. All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must 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 should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events

8.3.1 EC/IRB Notification by Investigator

Reports of all serious adverse events (including follow-up information) must be submitted to the Northwestern University Institutional Review Board (NUIRB0 within 10 working days. Copies of each report and documentation of NUIRB notification and receipt will be kept in the Clinical Investigator's binder.

8.4 Stopping Rules

If any of the following criteria are met, study enrollment will be suspended and participants will be maintained on their current IS therapy or may be required to restart IS therapy: 1) Rejection induced by IS weaning resulting in death, retransplantation or listing for retransplantation in any one subject; 2) Either steroid-resistant rejection or chronic rejection occurring during weaning in >10% of subjects (2 patients)

8.5 Medical Monitoring

The Principal Investigator (Dr. Levitsky) will oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Subjects will be assessed at their visits and during phone calls for Aes/SAEs.

9 Data Handling and Record Keeping

9.1 Confidentiality

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:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include; but are not limited to: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

An electronic case report form (eCRF) is the primary data collection instrument for the study.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years on site after the study has been terminated with the NUIRB. These documents should be retained off site up to 13 more years at the PIs discretion. If kept off site, the records will be stored at O'Hare Records, in Rosemont, IL.

9.5 Auditing and Inspecting

The investigator will permit study-related audits, and inspections by the NU IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related

facilities (e.g. clinic, diagnostic laboratory, etc.). As an investigator in this study acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices is expected.

10 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 parts 312, 50, 56 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the NU IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the NU IRB concerning the conduct of the study will be made in writing to the investigator.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. A copy of the Subject Informed Consent Form follows this protocol. This consent form will be submitted with the protocol for review and approval by the NU IRB for the study. The formal consent of a subject, using the NU IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator or investigator-designated research professional obtaining the consent.

11 Study Finances

11.1 Funding Source

Funding will be requested through a specific fund allocated to the transplant Hepatology group at Northwestern. Additional funding provided through an NIH grant.

11.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) will have the conflict reviewed by a properly constituted Conflict of Interest Committee. All Northwestern University investigators will follow the University conflict of interest policy.

11.3 Subject Stipends or Payments

None

12 Publication Plan

Any and all scientific, commercial and technical information disclosed by the Principal Investigator (sponsor) in this protocol or elsewhere should be considered the confidential proprietary property of the PI and the Comprehensive Transplant Center (CTC).

The results of this study may also be used for teaching, publications, and/or presentations at scientific meetings. If individual results are discussed, the identity of the subjects will be protected by use of a study code number.

13 References

1. Gonwa TA, Mai ML, Melton LB, Hays SR, Goldstein RM, Levy MF, et al. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTX) using calcineurin-based immunotherapy: risk of development and treatment. Transplantation. 2001;72(12):1934-9.
2. Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, et al. Chronic renal failure after transplantation of a nonrenal organ. N Engl J Med. 2003;349(10):931-40.
3. Morard I, Dumortier J, Spahr L, Hadengue A, Majno P, Morel P, et al. Conversion to sirolimus-based immunosuppression in maintenance liver transplantation patients. Liver Transpl. 2007;13(5):658-64.
4. Fairbanks KD, Eustace JA, Fine D, Thuluvath PJ. Renal function improves in liver transplant recipients when switched from a calcineurin inhibitor to sirolimus. Liver Transpl. 2003;9(10):1079-85.
5. Starzl TE. The "privileged" liver and hepatic tolerogenicity. Liver Transpl. 2001;7(10):918-20.
6. Levitsky J. Operational tolerance: past lessons and future prospects. Liver Transpl. 2011;17(3):222-32.
7. Battaglia M, Stabilini A, Roncarolo MG. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. Blood. 2005;105(12):4743-8.
8. Turnquist HR, Raimondi G, Zahorchak AF, Fischer RT, Wang Z, Thomson AW. Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic CD4+ T cells, but enrich for antigen-specific Foxp3+ T regulatory cells and promote organ transplant tolerance. J Immunol. 2007;178(11):7018-31.
9. Baan CC, van der Mast BJ, Klepper M, Mol WM, Peeters AM, Korevaar SS, et al. Differential effect of calcineurin inhibitors, anti-CD25 antibodies and rapamycin on the induction of FOXP3 in human T cells. Transplantation. 2005;80(1):110-7.
10. Szabo G, Gavala C, Mandrekar P. Tacrolimus and cyclosporine A inhibit allostimulatory capacity and cytokine production of human myeloid dendritic cells. J Investig Med. 2001;49(5):442-9.
11. Gao W, Lu Y, El Essawy B, Oukka M, Kuchroo VK, Strom TB. Contrasting effects of cyclosporine and rapamycin in de novo generation of alloantigen-specific regulatory T cells. Am J Transplant. 2007;7(7):1722-32.
12. Levitsky J, Miller J, Wang E, Rosen A, Flaa C, Abecassis M, et al. Immunoregulatory profiles in liver transplant recipients on different immunosuppressive agents. Human Immunology. 2009;70(3):146-50.
13. Levitsky J, Mathew JM, Abecassis M, Tambur A, Leventhal J, Chandresekaran D, Herrera N, Al-Saden P, Gallon L, Abdul-Nabi A, Yang GY, Kurian S, Salomon D, Miller J. Systemic Immunoregulatory and Proteogenomic Effects of Tacrolimus to Sirolimus Conversion in Liver Transplant Recipients. Hepatology 2012;(in press).

14. Levitsky J, Gallon L, Miller J, Tambur AR, Leventhal J, Flaa C, et al. Allospecific regulatory effects of sirolimus and tacrolimus in the human mixed lymphocyte reaction. Transplantation. 2011;91(2):199-206.
15. Mazariegos GV, Zahorchak AF, Reyes J, Ostrowski L, Flynn B, Zeevi A, et al. Dendritic cell subset ratio in peripheral blood correlates with successful withdrawal of immunosuppression in liver transplant patients. Am J Transplant. 2003;3(6):689-96.
16. Martinez-Llordella M, Lozano JJ, Puig-Pey I, Orlando G, Tisone G, Lerut J, et al. Using transcriptional profiling to develop a diagnostic test of operational tolerance in liver transplant recipients. J Clin Invest. 2008;118(8):2845-57.
17. Levitsky J, Mathew JM, CW Flaa, A Rosen, AR Tambur, J Miller. The human "Treg MLR": Immune monitoring for FOXP3+ T Regulatory Cell Generation. Transplantation. 2009 Dec;88(11):1303-11.
18. Mathew JM, Blomberg B, Fuller L, Burke GW, Ciancio G, Kenyon N, et al. A novel micro-cell-mediated lympholytic assay for the evaluation of regulatory cells in human alloreactive CTL responses. Journal of Immunological Methods. 2003;272(1-2):67-80.
19. Levitsky J, Leventhal JR, Miller J, Huang X, Chen L, Chandrasekaran D, et al. Favorable effects of alemtuzumab on allospecific regulatory T-cell generation. Hum Immunol. 2011.
20. Yu Y, Miller J, Leventhal JR, Tambur AR, Chandrasekaran D, Levitsky J, et al. Requirement of cognate CD4+ T-cell recognition for the regulation of allospecific CTL by human CD4+ CD127- CD25+ FOXP3+ cells generated in MLR. PLoS One. 2011;6(7):e22450.
21. Levitsky J, Salomon DR, Abecassis M, Langfelder P, Horvath S, Friedewald J, et al. Clinical and plasma proteomic markers correlating with chronic kidney disease after liver transplantation. Am J Transplant. 2011;11(9):1972-8.