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Official Title:	Efficacy of Low Dose, SubQ Interleukin-2 (IL-2) to Expand Endogenous Regulatory T-Cells in Liver Transplant Recipients
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PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	Efficacy of Low Dose, Subcutaneous Interleukin-2 (IL-2) to Expand Endogenous Regulatory T-Cells in Liver Transplant Recipients
Principal Investigator	**Michael Curry, MD**

B1. PURPOSE OF PROTOCOL

The primary objectives of this pilot study are:

1. To assess the efficacy of very low dose subcutaneous interleukin-2 (IL-2) to promote regulatory T cell (Treg) expansion in a cohort of liver transplant recipients with stable graft function, no active or history of auto-immune diseases, no evidence of allograft rejection, no current evidence of viral hepatitis, and no advanced liver graft fibrosis.
2. To asses the safety and tolerability of very low dose IL-2 in a liver transplant recipient cohort.

Secondary objectives:

1. To investigate the percent change of peripheral blood Treg cells and change in phenotype and function of Treg cells in response to very low dose IL-2 in a liver transplant recipient cohort.
2. To identify patients who are IL-2 responsive and thereby potential candidates for withdrawal of immunosuppression. This pilot study will not involve withdrawal of immunosuppression (IS), however if it is determined that low dose IL-2 is safe, well tolerated, and results in significant expansion of Treg cells, then subsequently a follow-up study of IL-2 expansion of Treg cells followed by IS withdrawal will be proposed.
3. Assess short term impact of study treatment on subject's DSA status, i.e. change in DSA titers or development of de novo DSA.

Exploratory Objective:

1. Establish baseline phenotypic values for T cell exhaustion in the trial cohort.
2. Determine if IL-2 treatment results in development of IL-2 antibodies in the study population

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Transplantation remains the most successful treatment for end-stage liver disease (ESLD), but the need to administer life-long immunosuppression (IS) to prevent rejection limits patient and graft survival. Systemic immunosuppression is associated with the development of significant morbidity from complicating side effects of hypertension, hyperglycemia and hyperlipidemia contributing to the metabolic syndrome and cardiovascular disease, cerebrovascular disease and renal failure. Additionally, long-term use of immunosuppression is associated with a remarkably increased risk of skin and other malignancies. Long-term survival following liver transplantation is still suboptimal, and liver recipients exhibit higher morbidity and mortality than the general population. This is mainly due to co-morbidities arising in large measure from chronic IS treatment.

Liver transplantation is the only organ transplant setting in which a sizeable proportion of patients spontaneously develop "operational tolerance", a phenomenon defined by the maintenance of stable graft function in the absence of destructive immune responses without the need of IS. Withdrawal of IS is feasible in selected patients who develop spontaneous operational tolerance, and may overcome the problems of exposure to long term IS. Unfortunately, this phenomenon only occurs many years after transplantation (i.e. 79% of adult patients >11 years post-transplant, but in 0% of adult patients <50 years old and <6 years post-transplant), when irreversible IS-related toxicity has

often already occurred. Therefore, there is an urgent need to develop strategies to intentionally induce tolerance in young liver recipients who have been transplanted for a short period of time.

Immunological tolerance is regarded as an active regulatory process whose outcome depends on the balance between cytopathic lymphocytes and graft-protecting, regulatory T cells (Tregs). In animal models of transplantation, liver allografts are spontaneously tolerated through regimens that dependent on CD4+CD25+Foxp3+ T cells (Tregs). In human liver recipients undergoing IS withdrawal years after transplantation, transient intra-hepatic lymphocyte infiltration with preferential accumulation of Tregs is also observed. This suggests that a shift in the balance between regulatory and effector T cells is critical to achieve tolerance, and that IS withdrawal provides a window of opportunity to do so.

IL-2 is essential for the survival and function of Tregs. In animals, inefficient IL-2 signalling results in Treg deficiency and autoimmunity, whereas administration of IL-2 in vivo induces Treg expansion and immunoregulation. Deficient IL-2 signaling is a hallmark of human autoimmune diseases such as type-1 diabetes. Calcineurin inhibitors (CNI), which constitute the mainstay IS in liver transplantation, reduce the overall availability of IL-2. Studies have determined that in liver recipients on CNI, Tregs are reduced in number as a consequence of increased apoptosis. Low-dose IL2 reverses this phenotype by upregulating the anti-apoptotic BCL-2 gene selectively in Tregs thereby promoting Treg expansion. Tregs, cells that bear high affinity IL-2 receptors, require IL-2 to expand and maintain their immunosuppressive phenotype. Tregs not only expand in blood, but also in peripheral tissues, with the largest increase being observed in the liver.

Recent clinical studies using low dose IL-2 in patients with drug resistant GVHD and certain forms of autoimmunity demonstrated that low doses IL-2 (1 million units/m2) is safe and efficacious with a 50% response rate in GVHD. Clinical studies performed to date show that even very low doses of IL-2, i.e., 0.3 million units/m2, expands Tregs whereas doses of 1million units/m2 also expanded the number of pro-inflammatory innate immune cells bearing intermediate affinity IL-2Rs.Expansion of pro-inflammatory cells could counteract, to some degree, the immunoregulatory effects of IL-2 driven expansion of Tregs. Therefore, this lower dose (0.3 million IU/m2) would potentially limit this unwanted consequence and boost both efficacy and safety. The affinity of Tregs for IL-2 is much higher than innate immune cells except for a tiny, desirable population of highly IL-2 responsive immunoregulatory NK cells. Additioanlly, in recent studies of cynomolgus monkey renal transplant recipients, with durable drug free graft survival, rejection was incited by daily treatment with 1 million IU/ m2. IL-2 induced an unwanted expansion of memory T cells. Taken together, the results in clinical and monkey studies indicate that in treatment with 1 milion IU/ m2 IL-2, levels of IL-2 hover in a range where Tregs expand, but this is accompanied by unwanted activation of innate immune cells and/or T-memory, such that the stimulatory effect on Tregs is offset, sometimes moderately, sometimes markedly, by activation and expansion of inflammatory innate and memory T cells.

In consideration of these most recent findings, we amend the protocol (version date 9.7.2017), to reduce the dose of IL-2 (from 1.0 million to 0.3 million units/m2) to expand Tregs, at a dose that does not expand pro-inflammatory immune cells to further improve the safety profile while still promoting tolerance.

This provides additional rationale for administering a very low-dose IL-2 to liver transplant recipients to expand the pool of endogenous Tregs and shift the balance between effector and regulatory lymphocytes at the time IS drugs are discontinued.

To maximize the benefit derived from IS withdrawal, there is a need to find strategies to intentionally induce tolerance in young recipients in whom accumulated IS toxicity has not yet occurred. Tregs can suppress cytopathic immune responses and inhibit transplant rejection. In contrast to innate immune cells and effector T cells, resting Tregs constitutively express the high affinity IL-2 receptor, making Tregs exquisitely sensitive to very low-doses of IL-2. This protocol proposes to employ low-dose IL-2 to promote the selective expansion of endogenous Tregs and shift the balance between effector T cells and Tregs. Studies have revealed that successful IS discontinuation is associated with a

transient intra-graft immune regulatory response with preferential accumulation of Treg cells. This suggests that short-term enhancement of Treg numbers and/or function at the time of IS withdrawal may facilitate the acquisition of tolerance in patients who are not predisposed to spontaneously develop it. IL-2 is a cytokine that is essential for the optimal development, survival and function of Tregs. Several clinical studies have shown that low-dose IL-2 preferentially expands Tregs and is safe and efficacious in patients with autoimmunity or graft verses host disease (GVHD). In these studies, Treg frequency increased up to 2 to 8-fold without significant changes in the number of effector T cells. Our objective is to investigate if administration of a short-course of low-dose IL-2 to liver transplant recipients facilitates an increase in peripheral blood Treg cells. We propose to conduct this pilot study to assess safety and efficacy of IL-2 in expanding peripheral blood Treg cells and assess the effect of IL-2 on T effector cells and cell of the innate immune response in these transplant recipients.

The primary objectives of this pilot study are:

1. To assess the efficacy of very low dose subcutaneous interleukin-2 (IL-2) to promote regulatory T cell (Treg) expansion in a cohort of liver transplant recipients unlikely to be safely withdrawn from from IS in the absence of other treatment with stable graft function, no active or history of auto-immune diseases, no evidence of allograft rejection, no current evidence of viral hepatitis, and no advanced liver graft fibrosis.
2. To asses the safety and tolerability of very low dose IL-2 in a liver transplant recipient cohort;
3. Assess short term impact of study treatment on subject's DSA status, i.e. change in DSA titers or development of de novo DSA.

Secondary objectives:

1. To investigate the percent change of peripheral blood Treg cells and change in phenotype and function of Treg cells in response to very low dose IL-2 in a liver transplant recipient cohort;
2. To identify patients who are IL-2 responsive and thereby potential candidates for withdrawal of immunosuppression. This pilot study will not involve withdrawal of immunosuppression (IS), however if it is determined that very low dose IL-2 is safe, well tolerated, and results in significant expansion of Treg cells, then subsequently a follow-up study of IL-2 expansion of Treg cells followed by IS withdrawal will be proposed.

Exploratory objective:

1. Establish baseline phenotypic values for T cell exhaustion in the study cohort.
2. Determine if IL-2 treatment results in development of IL-2 antibodies in the study population

References:

Benítez C. Multicenter clinical trial of immunosuppression withdrawal in liver transplantion. Hepatology 2013;58(5):1824-35

Bohne F. Intra-graft gene expression predicts the development of tolerance in liver transplantation. J Clin Invest 2012;122(1):368-82

Hartemann A. Low-dose interleukin-2 in patients with type-1 diabetes. Lancet Diabetes Endocrinol 2013 Dec;1(4):295-305.

Koreth J. Interleukin-2 and Regulatory T Cells in GVHD. N Engl J Med. 2011;365(22):2055-66

Matsuoka K. Interleukin-2 restores regulatory T cell homeostasis in chronic GVHD. Sci Transl Med 2013;5(179):179ra43

Yamada Y, Nadazdin O, Boskovic S, Lee S, Zorn E, Smith RN, Colvin RB, Madsen JC, Cosimi AB, Kawai T, Benichou G. Repeated Injections of IL-2 Break Renal Allograft Tolerance Induced via Mixed Hematopoietic Chimerism in Monkeys. Am J Transplant. 2015 Dec 1;15(12):3055–3066.

B3. DESCRIPTION OF RESEARCH PROTOCOL

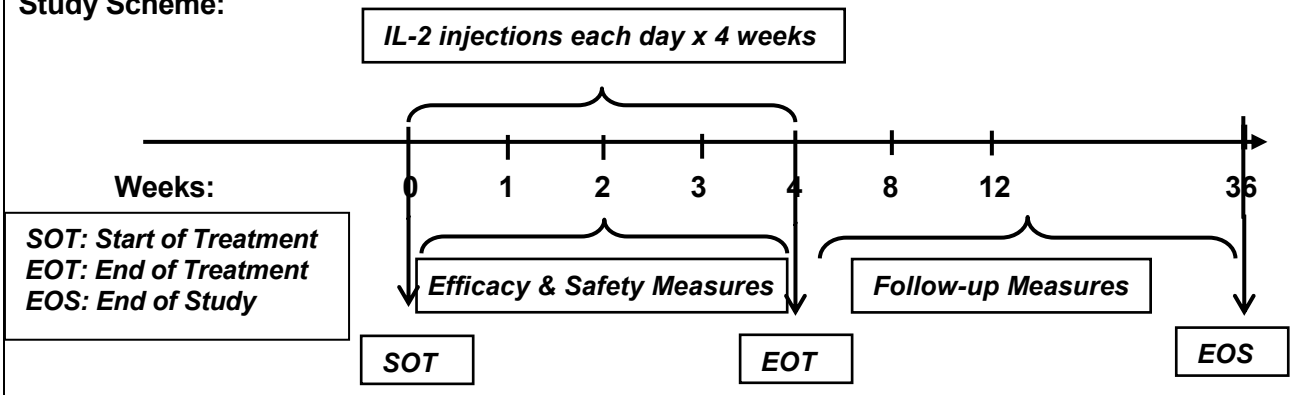
A. Study Design – Overview, Methods, Procedures

The study is a single arm, prospective, open label efficacy trial of a 4 week course of low dose subcutaneous IL-2 (1MIU/mL/m^2/day) to expand peripheral blood regulatory T cells (Tregs) in a liver transplant population..

This pilot trial will treat up to 10 liver transplant recipients who are ≤65 years of age, a minimum of 2 years but no more than 4 years post primary liver transplantation for non-autoimmune conditions, have had no evidence of transplant rejection, and have no current evidence of liver allograft dysfunction or viral hepatitis.

Study objectives include (1) determine the efficacy of the administration of low dose IL-2 to promote Treg cell expansion; (2) asses the safety and efficacy of low dose IL-2 in a liver transplant recipient cohort; (3) investigate the percent change of peripheral blood Treg cells and change in phenotype and function of Treg cells in response to low dose IL-2 in a liver transplant recipient cohort.

Study Scheme:



Study Procedure:

Screening (S0):

After obtaining informed consent the following screening procedures will be completed:

- Full history and medical record review
- Physical examination including height and weight
- Vital signs (blood pressure, heart rate and temperature)
- Blood (approximately 20 milliliters) will be drawn for
  - ✓ liver function panels (AST, ALT, ALP, Bilirubin, Albumin) and kidney function panels (Na, K, Cl, CO2, Creatinine, Glucose, Ca) if not previously obtained within 1 month of screening
  - ✓ viral hepatitis and autoimmune panels (HBV sAg, HCV Ab, ANA, ASMA IgG) if not previously obtained within 6 months of screening
  - ✓ serum pregnancy test for women of child bearing potential (WOCBP)
- Fibroscan if liver biopsy/fibroscan results not obtained within 1 year of screening
- ECG if not obtained within 3 months of screening

Subjects with liver panel results <2xULN, serum creatinine <1.5 ULN; no evidence of viral hepatitis or

autoimmunity, no evidence of pregnancy, absence of advanced liver fibrosis, no history of seizure disorder and no evidence of cardiac disease will proceed with the study (see Inclusion/Exclusion criteria in section below).

Baseline/Treatment Visit Day 1 (W0):

Subjects will return to clinic to complete baseline measures and begin study treatment:

- Review of any new health problems, current medication and vital sign measures;
- Blood draw (approximately 35 milliliters) for
  - ✓ CBC with differential,
  - ✓ liver and kidney function panels,
  - ✓ immune cell counts ,
  - ✓ T cell exhaustion phenotyping,
  - ✓ serum IL-2 levels,
  - ✓ CNI trough levels,
  - ✓ presence of donor specific antibodies (DSAs)
  - ✓ presence of anti-IL-2 antibodies

Treatment: Subjects will start very low dose **IL-2 subcutaneous injections at a once per day dose of 0.3 million IU/mL per square meter body surface area (BSA) for 4 weeks**. Study staff will instruct subjects and/or identified care providers (e.g. friend or spouse) on subcutaneous injection technique.

The first study dose will be administered in the BIDMC Harvard-Thorndike Clinical Research Center. Subjects will remain in house for 2 hours of observation to monitor drug tolerability and adverse events.

Subjects will be given a paper calendar to record their self-administration of the study drug. Subjects will record the time of injections each day and bring the study calendar and all unused drug to all study visits. (see Calendar attached)

Treatment (D3):

Subjects will return to clinic on Day 3 for evaluation of adherence to treatment and staff observation of proper injection technique (for Dose 3) and the following measures will be obtained:

- Review of any new health problems, current medications
- Vital signs
- Blood draw (approximately 10 milliliters) for liver and kidney function panels

Treatment (W1, W2, W3, W4)

Subjects will return to clinic weekly until Day 28 (Week 4). Subjects will receive a refill of IL-2 (not at Week 4 visit) and the following measures will be obtained:

- Review of any new health problems, current medications, vital signs and weight,
- ECG,
- Blood draw (approximately 35 milliliters) will be drawn for
  - ✓ CBC with differential,
  - ✓ liver and kidney function panels,

- ✓ serum IL-2 levels,
- ✓ immune cell counts (Weeks 2 and 4)
- ✓ Week 4 only: CNI trough levels and serum pregnancy test (WOCBP)
- ✓ DSAs (Week 4)
- ✓ anti-IL-2 antibodies (Week 4)

Subjects will discontinue IL-2 treatment after Day 28 (Week 4).

Subjects will have blood drawn for standard of care labs at Week 4, i.e. CBC, liver and kidney panels, and CNI trough levels. We will obtain standard of care results from subjects' medical records and draw extra blood (approximately 25 ml) for research testing for DSA and anti-IL-2 antibodies (week 4), serum IL-2 levels and PBMCs flow cytometry and a serum pregnancy will be conducted for WOCBP.

Follow-up (W8, W12, W36)

Subjects will return to clinic for post-treatment follow-up at Weeks 8, 12 and 36. The following measures will be obtained:

- Review of any new health problems, current medications, vital signs and weight,
- Full physical examination will be completed at end of study (Week 36);
- Review standard of care lab results for
  - ✓ CBC with differential,
  - ✓ liver and kidney function panels,
  - ✓ CNI trough levels,
- Blood draw (approximately 20 milliliters) for study labs
  - ✓ Immune cell counts (Weeks 8 and 12)
  - ✓ serum IL-2 levels (Weeks 8 and 12)
  - ✓ serum pregnancy test for WOCBP (Weeks 8 and 12).
  - ✓ anti-IL-2 antibodies (Week 36)

Dosage Reduction and Treatment Withdrawal

The dose of IL-2 may be reduced by half to 0.15 million IU /m^2 daily if a subject experiences untoward side effects. If symptoms improve, the dose may be re-escalated to target. If symptoms persist, the dose frequency may be reduced to every other day administration or the Investigator may discontinue study treatment.

Study treatment will be discontinued in the presence of any of the following circumstances:

- ✓ Subject withdraws consent
- ✓ Life threatening anaphylactic reaction to IL-2
- ✓ Life threatening infection on IL-2
- ✓ The Investigator may discontinued study treatment in the presence of other clinically significant findings including abnormal ECG, blood panel results >2x ULN and/or other signs of significant toxicities.

Subjects who are withdrawn from treatment will complete remaining study visits and continue to receive follow-up care from his/her BIDMC transplant Hepatologist.

Standard of Care Maintenance Immunosuppression: Eligible patients must be receiving stable dosing of immunosuppressant therapy for a minimum of 1 month prior to study enrollment. Maintenance immunosuppression is customize to the patient but typically, stable liver transplant patients at 2-4 year post transplant who are otherwise eligible to enroll into this trial would be taking tacrolimus (target 12 hour trough goal of 4-6ng/mL) with or without mycophenolic acid (as mycophenolate mofetil or mycophenolate sodium) at a dose between 500-1000mg twice a day.

Safety Monitoring

The PI is ultimately responsible for protecting the rights, safety and welfare of subjects enrolled into the trial. Study visits will be conducted weekly during the treatment phase of the trial; post treatment follow-up visits will be conducted at week 8 and 12. The PI will review all safety parameters including clinical safety laboratory assessments within 48 hours of completion of the measure. Clinical laboratory values that fall outside of the pre-specified safety ranges will be brought to the PIs attention the day the reports are final to assess clinical significance.

Patients of the Transplant Institute have direct access to Transplant Nurse Coordinator consultation 24/7/365. Transplant Coordinators will alert the PI or designee for any study subject who calls with questions or concerns.

Patients will be given a Study Information card and instructed to present it to any health care provider they see during the time that are participating in the trial. The Study ID card will include a brief description of the trial, name of the study drug, and PI contact information. See safety card attached.

10.2 Monitoring for AEs

Subjects will be monitored for the occurrence of adverse events at all study visits, including follow up.

Reporting procedures will comply with BIDMC IRB policies; US Federal Regulations (21CFR 312), and ICH Guideline E-6: *Guidelines for Good Clinical Practice.*

Adverse events will be coded and graded according to the NIH/NCI Common Terminology Criteria for Adverse Events version 4.03 (June 2010). All adverse events will be classified on seriousness (mild, moderate, serious), expectedness (expected, non-expected), and relatedness to trial (not related, possibly related, related).

Reporting Serious Adverse Events: All serious adverse events (SAEs) will be reported to the BIDMC IRB, regardless of the determined relationship to the study drug, within the timeframes specified below:

- **Unexpected Fatal or Life-Threatening SAEs** will be reported within one (1) business day of PI awareness. A formal written report (signed by the PI) will be submitted within seven (7) calendar days of PI awareness.
- All **other unexpected SAEs** will be reported as a formal written report (signed by the PI) within seven (7) calendar days of PI awareness.
- **Expected SAEs**: For all expected SAEs, we will submit a formal, written report (signed by the PI) with fourteen (14) calendar days of PI awareness.

Non-serious AEs will be evaluated by the PI to determine the relationship of the event to the study drug, then reported to the CCI in the timeframe and manner specified below:

- **Related**: Non-Serious AEs determined by the PI to be possibly, probably, or definitely related to the Study (or relationship unknown):

- ✓ Unexpected/related: non-serious AEs that are unexpected and determined to be related to study drug, will be reported in a formal written report (signed by the PI) within fourteen (14) calendar days of PI awareness.
- ✓ Expected/related: non-serious AEs that are expected and determined to be related but which occur with greater frequency than expected, will be summarized for submission on the continuing review progress report at the study's continuing review.

- **Unrelated**: Non-serious AEs determined by the PI to be unrelated to the study:
  - ✓ Unexpected /unrelated: non-serious AEs that are unexpected and determined to be unrelated which occur with greater frequency than expected, will be summarized for submission on the continuing review progress report at the study's continuing review

IND Safety Reporting

In compliance with federal regulation (21 CFR 312), safety reports will be submitted to the FDA (via submission of an IND Safety Report) when an event meets all of the following criteria:

- Suspected adverse reaction (reasonable possibility of relationship with study drug)
- Serious
- Unexpected

Fatal or life threatening events will be reported as soon as possible but no later than 7 calendar days from time the Sponsor becomes aware.

Serious and unexpected adverse events Adverse Events will be reported as soon as possible but no later than 15 days from the time the Sponsor becomes aware.

Reporting to Prometheus

A copy of any SAE report submitted to the BIDMC IRB, Sponsor and FDA will be sent to Prometheus Laboratories. If interleukin-2 is a suspect or co-suspect drug reported on the FDA Form 3500A MedWatch Report, a copy of the FDA Form (3500A MedWatch Report) will also be sent to Prometheus Laboratories as a courtesy. Reports to Prometheus will be sent to Drug Safety and Pharmacovigilance at Prometheus Laboratories Inc. All contact information will be included in the Report.

Drug Safety Email: drugsafety@prometheuslabs.com
Drug Safety fax: (858) 754-3046

Avoiding bias in laboratory testing:

Blood specimens drawn and processed for immunological testing will be de-identified, logged and labeled prior to transfer to the central laboratory. Specimen labeling system will be designed such that the lab staff will not be aware of the visit sequence of the specimen in relation to pre vs post treatment status.

Independent Medical Monitor (IMM)

The PI has named an Independent Medical Monitor who is familiar with the agent and with the patient population, Simon Robson, MD, PhD, Professor of Medicine, Harvard Medical School. Dr. Robson is a world leader in the study of vascular and immune cell ectonucleotidases in pathophysiology and transplantation. He is also a prominent investigator in liver and gastrointestinal diseases.

The IMM will review all SAE reports sent to the BIDMC IRB, Sponsor and FDA in real time. The IMM will also review aggregate data as they are available.

Routine Monitoring: To further ensure the study is conducted and documented in accordance with the investigational plan, applicable regulations and good clinical practices, routine monitoring will be conducted by the Transplant Institute's Clinical Research Associate or other qualified designee in fulfillment of the Sponsor's obligation for monitoring. Monitoring activities will include:

- Review of essential documents in the regulatory binder for completeness and currency;
- Inspection of every (100%) informed consent form to assure that correct versions are used; that the names of subject and consenter are legible; that forms are properly signed and dated by subject and consenting physician investigator; and documentation is available to document consent procedures.
- Verification that every enrolled subject meets eligibility criteria;
- Review of a minimum of 10% of case report forms entered to source verify accuracy and completeness with specific attention to …
    - ✓ Dose modifications
    - ✓ Adverse events, concomitant medications and intercurrent illnesses
    - ✓ Missed study visits, procedures, labs, etc
    - ✓ Drop out and withdrawals
- Review of all regulatory reporting
- Reporting findings to Sponsor/Investigator.

Table 1: Study Visits Schedule and Procedures

	Screening and Baseline		*Treatment to Week 4*					*Follow-up to Week 36*		
Visit	*Screening*	*Baseline Day 1*	*Day 3*	*W1*	*W2*	*W3*	*W4*	*W8*	*W12*	*W36*
Study Procedures: *Visit #*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Informed Consent	√									
Full Physical Exam & Medical History	√									√
Limited Physical Exam & Medical History		√	√	√	√	√	√	√	√	
Vital Signs	√	√	√	√	√	√	√	√	√	√
Electrocardiogram (ECG)	√			√	√	√	√			
Fibroscan	√[1]									
Study Blood Tests:										
Pregnancy test (for women of child bearing potential)	√						√	√	√	
CBC with Differential		√	√	√	√	√	√[4]	√[4]	√[4]	√[4]
Liver panel (AST, ALT, AP, Total Bilirubin, Albumin)	√[2]	√	√	√	√	√	√[4]	√[4]	√[4]	√[4]
Kidney panel (Na, K, Cl, CO2, Creatinine, Glucose, Ca)	√[2]	√	√	√	√	√	√[4]	√[4]	√[4]	√[4]
Viral hepatitis and autoimmunity panels (HBVsAg, HCVAb, ANA, ASMA IgG)	√[3]									
Immunosuppression drug level		√					√[4]	√[4]	√[4]	√[4]
Immune cell counts		√			√		√	√	√	
IL-2 Blood level		√		√	√	√	√	√	√	
T-cell exhaustion markers (only at baseline)		√								
DSA (pre and post IL-2 treatment)		√					√			
Samples anti IL2 antibody testing		√					√			√

[1] *Only if a fibroscan or a liver biopsy was not completed for standard of case within the last 12 months*
[2] *Only if liver panel or kidney panel was not collected for standard of care within the last month*
[3] *Only if viral and autoimmunity tests not completed for standard of care within past 6 months*
[4] *Obtained as part of standard of care*

B. Statistical Considerations

Sample Size Justification: This is a pilot study to determine the efficacy and safety of very low dose IL-2 administration in a select group of liver allograft recipients. Investigators will consent as many as 12 subjects into the screening phase in order to treat not more than 10 patients from the BIDMC Transplant Institute's Liver Transplant Program.

The primary endpoint for the trial is the percent change in absolute numbers of regulatory T cells (Tregs) from pre-treatment baseline to end of treatment, i.e. the week 4 treatment visit.

For IL-2 to be clinically viable, we would require evidence that 60-80% of participants experience a biological response defined as a 2 fold increase in absolute number of Treg cells from baseline. Less than 50% of responding participants would be an unacceptable low response. The success of IL-2 will be defined based on a one sided 95% confidence interval (CI). The CI for a 0.80 response rate with 10 subjects would be 0.59-1.00 enabling exclusion of a response rate of 0.50.

Data Analysis: Differences in absolute numbers of circulating endogenous Treg cells from baseline to end of treatment will be compared using standard descriptive statistics and paired t-tests.

C. Subject Selection

Study subjects will be identified from the BIDMC Transplant Institute Liver Transplant Program by hepatologist Michael Curry, MD (Medical Director of the Liver Transplant Program) and surgeon Khalid Khwaja, MD (Surgical Director of the Liver Transplant Program).

Inclusion criteria:

1. Adult liver transplant recipients 2-4 years post trapslantation
2. Male or female, age ≤ 65 years old
3. Stable dosage of immunosuppressant therapy for 1 month prior to study.

Exclusion crtieria:

1. Recipient of multiple transplants (including solid organ, stem-cell, and bone marrow)
2. Serum liver panel (ALT, AST, Alkaline Phosphatase and Total Bilirubin) > 2 x ULN,
3. Serum creatinine > 1.5 x ULN,
4. eGFR of < 40 ml/min,
5. Detectable hepatitis viral load,
6. Abnormal ECG with clinically significant findings per study physician's judgement,
7. Active infection,
8. Presence or history of autoimmunity disorders,
9. Evidence of allograft rejection,
10. Liver biopsy or fibroscan evidence of advanced stage liver fibrosis (> Stage 2 Fibrosis),
11. Presence or history of cardiac or pulmonary disease,
12. Pregnant or nursing (lactating) women,
13. Presence or history of seizure disorder,
14. Health condition precludes participation in trial at study physician's judgment,
15. Inability to give consent.

Male and female participants of all racial distributions are eligible for this study. Participation is limited to subjects who are less than 4 years post-transplant and less than or equal to 65 years old as individuals who are further out from transplant and older in age are likely to have a higher chance of spontaneous operational tolerance and less likely to need pharmacologic intervention to achieve tolerance.

B4. POSSIBLE BENEFITS

It is not possible to predict whether subjects will benefit directly from participation in this study. Regardless, participation may help others in the future as a result of knowledge gained from the research.

Demonstration that very low dose, subcutaneous IL-2 can induce endogenous Treg cell expansion will allow further study of IL-2 mediated operational tolerance in liver allograft recipients. Future studies will consider reducing or withdrawing systemic IS in a cohort of patients who respond to IL-2 expansion of Tregs.

This preliminary study may identify patients who are IL-2 responsive and possible candidates for future studies of IS reduction. Successful induction of tolerance and withdrawal of IS would decrease the morbidity associated with lifelong use of IS agents for control of organ rejection.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

Risk of Interluekin-2 (Proleukin, IL-2):

IL-2 is approved as a treatment for metastatic renal cell carcinoma and metastatic malignant melanoma. It is most commonly administered to cancer patients as a high-dose intravenous treatment regimen. For these cancer indications, dosing guidelines prescribe a three times per day intravenous infusion of 600,000 International Units (IU) per kilograms of body weight (e.g. dose for a person weighing 82kg is 49.2 million IU x 3 infusions/day = 147.6 million IU per day, compared to a daily dose for the same person for the proposed protocol would = 1.95 million IU/day) Significant side effects are expected with the high dose IV regimen for cancer treatment though they are reversible once treatment is stopped. Type and severity of side effects is dose dependent.

The drug manufacturer Novartis has conducted 7 Phase I/II clinical trials in 235 patients with a variety of doses and schedules using subcutaneous IL-2. Dosages have ranged from 2 million IU per day to 9 million IU per day. The study populations included metastatic renal cell carcinoma, advanced malignancy, and chronic lymphocytic lymphoma. In these trials, the following adverse events were recorded:

- Pyrexia (fever) – 77%
- Rigors (shivering) – 68%
- Asthenia (tiredness) – 49%
- Anorexia (loss of appetite) – 54%
- Nausea – 50%
- Vomiting – 49%
- Diarrhea – 40%
- Headache – 35%
- Dry skin – 25%
- Rash – 24%
- Injection site inflammation – 24%
- Dyspnea (shortness of breath) – 24%
- Injection site reaction – 24%
- Cough – 23%
- Myalgia (muscle pain) – 23%
- Insomnia – 21%
- Hypokinesia (muscle rigidity) – 20 %
- Dizziness – 18%
- Abdominal pain – 16%
- Injection site pain – 14%

- Depression – 11%
- Pruritus (itchiness) – 10%
- Oliguria (decrease in urine production) – 9%
- Confusion – 8%
- Hypotension (low blood pressure) – 7%
- Anxiety – 6%
- Increased serum creatinine – 4%
- Tachycardia (increased heart rate) – 3%
- Gastrointestinal bleeding – 2%
- Arrhythmia (unusual heart beat)– 1%
- Myocardial infarction (heart attack) – <1%
- Kidney failure – <1%

The most common serious adverse event associated with ***high dosage, IV administered IL-2*** is capillary leak syndrome where capillary permeability increases and serum proteins and fluids move to the extravascular space. In subcutaneous administration of IL-2, capillary leak syndrome is much less frequent and less severe. In the Novartis clinical trials of ***subcutaneous IL-2***, no cases of pulmonary edema were recorded. Peripheral edema occurred in 14% of patients and unspecified edema occurred in 11% of patients.

IL-2 has not been approved for use for the indication being tested in this study. In this study we will test the efficacy and safety of low dose IL-2, i.e., 0.3 million IU per square meter body surface area (BSA) given once a day in subcutaneous injections, to liver transplant patients over a 4 week period of time to measure the effect on the number of peripheral Treg cells.

Low dose IL-2 has been tested in other clinical trials and similar adverse effects are expected in this trial. A dose escalation trial of IL-2 conducted at Dana Farber Cancer Institute tested safety and efficacy of low dose IL-2 at three dose levels, i.e., 0.3 million IU, 1 million IU, and 3.0 million IU (per body square meter per day) for treatment of chronic Graft vs Host Disease that was refractory to standard care (Koreth et al, 2011). IL-2 dose (0.3MIU/m2) for 3-4 weeks promoted the expansion of Tregs without expanding innate immune cells, however, study protocol required dose escalation to maximum tolerated dose and thus the 0.3 MIU/m2 dosing was not formally tested for clinical efficacy. 29 subjects were enrolled; one patient withdrew early. The following treatment related adverse events were reported in the 28 patients who could be evaluated:

- Induration (skin hardening) – 11%
- Thrombotic microangiopathy (kidney failure) – 7%
- Flu-like symptoms – 4%
- Increase in serum creatinine – 4%
- Thrombocytopenia (platelet deficiency) – 4%

Another clinical trial in Paris, France studied low-dose subcutaneous IL-2 at a dose of 1.5 million IU per day and a dose of 3 million IU per day in 10 Hepatitis C vasculitis subjects (Saadoun et al, 2011). No adverse events were reported at a dosage of 1.5 million IU per day and the following adverse events were reported at a dosage of 3 million IU per day:

- Injection site reaction – 50%
- Asthenia (tiredness) – 40%
- Flu like symptoms – 40%
- Myalgia (muscle pain) – 10%
- Hypertension (high blood pressure) – 10%
- Infection – 10%

Based on signs and symptoms observed in the first subject enrolled into this trial (2016P-00086), three adverse events are added to the list of expected adverse events, i.e. eosinophilia, mouth ulcer

and pharyngitis (sore throat).

Contraindications: Patients will be counselled not to undergo routine radiological studies that require the use of iodine containing contrast agents during the duration of the study – including follow up (8 weeks after final dose). There is a possible increased risk of adverse reaction to the contrast agents which include fever, chills, nausea, vomiting, pruritus, rash, diarrhea, hypotension, edema, and oliguria. Most reactions occur within 4 weeks of taking IL-2.

Patients will also be informed of the listed contraindication of use of high dose IL-2 in organ transplant patients when used for FDA approved treatments involving the higher dose IV administrations for metastatic melanoma and renal cell carcinoma. NB: While there is a theoretical risk of transplanted organ rejection with high dose IL-2 treatments, there have been no documented cases of organ rejection reported with use of IL-2.

Although the drug manufacturer recommends against use of high dose IL-2 for patients with organ transplantation, participants in this study will take low dose IL-2 as this study is testing if very low dose IL-2, taken by healthy liver transplant patients will increase the number of T-reg cells in the blood. If this study discovers that low dose IL-2 **does** increase T-reg cells, Investigators will plan a follow-up study to investigate if healthy liver transplant patients develop 'tolerance' for their transplanted liver. Patients who develop 'tolerance' may be candidates to decrease or discontinue anti-rejection medications in a follow up study.

Use of any live vaccination is contraindicated in patients taking IL-2. At every visit staff will review all concomitant medications with patients and patients will be instructed to report to study staff all concomitant drugs, including prescription drugs, vaccinations, over the counter medications, vitamins and supplements. Further, patients will be instructed to tell all other practitioners that they are participating in a clinical trial and show those practitioners the Study Information Card. Patients will also be instructed to check with the Study Doctor prior to taking any new prescribed medication, vaccination, OTC medication, vitamins or supplements.

PREGNANCY AND FERTILITY:

Because the effects of interleukin-2 on the developing fetus are not known, subjects may not participate in this study if they are pregnant or breastfeeding. Women capable of becoming pregnant will be required to take a pregnancy test to verify that they are not pregnant before receiving their first dose of IL-2.

Furthermore, women capable of becoming pregnant must agree to use adequate birth control for the duration of the study. For the purpose of this study, use of adequate birth control includes one of the following: oral hormonal contraceptives, implanted hormonal contraceptives, diaphragm with spermicide, Intrauterine device, condoms used with another form of contraception as listed above, and abstinence.

Men capable of fathering children must use adequate contraception while participating in this study. For the purposes of this study, adequate birth control means use of a condom and/or partner must use an approved method of birth control as listed above.

The effect of IL-2 treatment on fertility. It is also unknown what effect IL-2 may have on future pregnancies.

Study subjects who become pregnant while participating in this study must inform study investigators immediately. Subjects will be given a pregnancy test. If the results demonstrate that the subject is pregnant, the subject must withdraw from the study, and the study investigators will ask to monitor the pregnancy.

Risk of Blood Draw:

Blood samples are collected as a part of this research study. The risks involved in drawing blood from a vein may include, but are not limited to:

- Momentary discomfort where we draw the blood from,
- Bruising and swelling around the site where we draw the blood from,
- A feeling of lightheadedness when the blood is drawn, and
- Rarely an infection where we draw the blood from.

Risk of ECGs:

Subjects might experience temporary redness or a rash on the skin where the adhesive pads are placed.

Confidentiality:

There is the potential for loss of confidentiality by participating in this study. Every effort will be made to protect the confidentiality of subject's identifiable information.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment: Study participants will be identified from among the ranks of patients who receive post-transplant care in the BIDMC Liver Transplant Program. Once the protocol is approved, Drs. Curry and Khwaja will identify patients within the practice who meet preliminary eligibility criteria. Patients who meet preliminary criteria will be contacted by an Investigator to come to the TI to learn more about the trial, complete consent procedures and requisite screening measures to confirm eligibility.

Consent: Consent procedures will be completed for patients in a private setting, e.g. Transplant Center examination room, consultation room or Investigator's Office. A study investigator will review all study procedures, and risks and benefits will be discussed.

The patient will be given ample time to ask questions and all his/her questions will be answered. If the patient prefers to take the consent home to think about it and discuss participation with others, consent procedures will be completed at a subsequent visit.

Patients willing to consent to participation will be required to sign and date the BIDMC CCI approved consent; the Investigator completing consent procedures will also sign and date the consent document. The original consent will be maintained in the subject's research record, a copy will be placed in the subject's medical record, and a copy of the signed consent will be given to the subject.

Subject Protection: We do not believe participants are vulnerable to coercion or undue influence.

B7. STUDY LOCATION

Privacy: Consent will customarily be completed in an LMOB out-patient clinic exam room. In some instances, consent procedures may be completed in a hospital in-patient setting such as a hospital in-patient bedroom. Consent procedures will always be completed in a private setting beyond the earshot of uninvolved staff or other patients. Study measurements, special tests and blood and urine samples will be collected by BIDMC staff at BIDMC facilities where confidentiality and patient privacy is upheld through hospital policy. Only the minimal amount of information needed to accomplish the research purposes will be collected.

Physical Setting: Interactions with study participants will take place in a medical setting at BIDMC such as the CRC for first dosage observation and the Transplant Clinic in LMOB7 when patients come for standard of care visits. There may be occasions where study visits are completed in other clinical or hospital locations including a hospital in patient bedroom.

B8. DATA SECURITY

Study staff is trained in human subject protections regulation including hospital mandated CITI and

HIPAA standards. Access to clinical medical records used for the trial is strictly limited in accordance with hospital policies. Data abstracted from medical records will be managed in compliance with GCP clinical research best practices; both hard copy and electronic data collected for the investigation will be stored in restricted access files in the TI Clinical Research Offices and in electronic files and folders behind hospital firewalls. Data included in analytic files will be de-identified and the key linking data to patient identity will be maintained by the investigators in a separated restricted access location.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☒ No

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☒ No

B10 Dissemination of Research Results

The study investigators will inform participants directly of the results of their individual treatment response (responder or non-responder) to IL-2.

Study ID Card

PATIENT SAFETY CARD – PLEASE KEEP IN YOUR WALLET
I am participant in a clinical research study. Study treatment is 4 weeks daily low dose subcutaneous Proleukin® (recombinant IL-2), manufactured by Prometheus Laboratories.

Name: _____ DOB: _____

Address: _____ Phone: _____

Emergency Contact (1): _____ Phone: _____

Emergency Contact (2): _____ Phone: _____

Liver Transplant Patient – Beth Israel Deaconess Medical Center (BIDMC)

PATIENT SAFETY CARD – PLEASE KEEP IN YOUR WALLET

Study Treatment and Investigator Contact
Proleukin® Dose: $0.3x10^6$ units/m^2 SC daily (duration 4 weeks)
Physician Principal Investigator: Michael Curry, MD, BIDMC Transplant Institute.
You can contact him or an on call Transplant Nurse Coordinator 24/7

617-632-9700.

Study IRB Reference #: __2016P-000086_

Clinicaltrials.gov NCT# __NCT 02739412__

Calendar for Medication Recording

timeanddate.com

April 2016 (United States)

May 2016

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Click on Sign to add text and place signatures on a PDF file.

Sun	Mon	Tue	Wed	Thu	Fri	Sat
27 Easter Sunday	28	29	30	31 3rd Quarter	1	2
3	4	5	6	7 New Moon	8	9
10	11	12	13 Thomas Jefferson's Birthday	14 1st Quarter	15	16
17	18	19	20	21	22 Full Moon	23
24	25	26	27	28	29 3rd Quarter	30