Protocol Title: Safety and efficacy of treatment with ultra-low dose subcutaneous IL-2 to promote regulatory T cells post renal transplantation

Short Title: Low Dose IL-2 Study

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1. Background and Significance

Post-transplant immunosuppression and transplant survival

Conventional immunosuppressive drugs used in organ transplantation are non-specific and broadly suppress the immune response[1]. As a result, transplant patients commonly suffer from complications related to infection and malignancy. Furthermore, long-term exposure to immunosuppressive drugs is associated with significant toxicity. While recent advances in treatment have markedly improved short-term transplant outcomes, with graft survival rates >90% at one year, long term transplant survival has failed to similarly improve and is essentially unchanged in recent decades. Chronic immunologic injury or drug-related toxicity is thought to play a role in up to 50% of cases of late allograft failure.

The immunological response to a transplanted organ is determined by the balance between effector T cells, which mediate rejection, and regulatory T cells, which promote allograft acceptance and tolerance. As our understanding of this balance has grown in recent years, the field of transplantation is moving away from developing therapies that suppress aggressive effector responses in isolation. There is increasing focus on finding ways to combine this approach with strategies to promote the development of tolerance to the transplanted organ.

Regulatory T cells in transplantation

Regulatory T cells (Tregs) make up around 10% of peripheral CD4+ T cells and their function is to suppress the proliferation and function pro-inflammatory effector T cells. They are essential to the maintenance of healthy immune homeostasis and have been shown to be of crucial importance in animal models of transplantation tolerance[2-4]. In humans, increased numbers of circulating Tregs have been found in transplant recipients who are considered to be 'operationally tolerant', i.e. they have a functioning transplant without taking any maintenance immunosuppression[5]. Furthermore, there are increased percentages of Tregs in stable kidney transplant patients compared to those with chronic allograft dysfunction[3,6]. While the potential of Tregs to influence transplant immune homeostasis is extremely attractive, the translation into a clinical therapeutic strategy has been challenging. Many currently available immunosuppressive drugs non-specifically target all T cells and can have detrimental effect of the Treg population. There is an unmet need to develop new strategies which specifically promote Tregs post transplantation in an attempt to improve long term outcomes.

Recombinant IL-2

Tregs express high levels of the high affinity IL-2 receptor subunit, CD25. Studies have shown that IL-2 stimulation is necessary for the proper regulation of genes involved in cell cycle, growth and metabolism, therefore contributing to Treg homeostasis. Exogenous treatment with IL-2 enhances Treg expansion and survival *in-vivo* [7-10]. IL-2 has a short half-life, therefore administration of recombinant IL-2/IL-2 monoclonal antibody (rIL-2/IL-2 mAb) complexes has become a standard technique for systemic *in-vivo* expansion of Tregs [7].
High dose IL-2 has been used as an adjunctive chemotherapy for patients with metastatic melanoma and renal cell carcinoma. At the doses given for these indications, the strategy is to saturate and activate both low and high affinity IL-2 receptors, with the aim that the net effect will be an increase in anti-tumor immune activity[11] (ref). While this treatment has shown success in selected patients, it is hampered by high incidence of serious dose-related side effects from IL-2 therapy[12].
The use of low dose IL-2 as a growth factor specifically for Tregs cells has been shown to be effective in multiple settings, in both primate and human studies [13-18]. Daily low-dose IL-2 injections used in naïve cynomolgus monkeys showed markedly expanded Tregs with limited expansion of other T cells and natural killer (NK) cells [13]. The majority of these expanded Tregs were found to be significantly suppressive of T cell activation *in vitro*.
Human studies have assessed the safety of low-dose rIL-2 in participants with steroid-refractory chronic graft versus host disease (GVHD) post hematopoietic stem cell transplant (HSCT) [19]. In a landmark study published from a group at DFCI, the primary objective was to determine the maximum dose tolerated (MTD) and toxicity of an 8-week course of low-dose rIL-2 in patients with chronic GVHD who responded poorly to steroids. At a dose of $1x10^6$ IU/m^2/day recombinant IL-2, none of the patients experienced GVHD flare or malignant disease relapse. 12 of 23 participants had objective clinical responses. Low-dose rIL-2 selectively increased Treg counts *in-vivo* without impacting T effector counts. In this human study, NK cell counts rose, to a lesser extent than Tregs, but low-dose IL-2 did not impact other T cell populations. Treg count remained elevated at 8 weeks of IL-2 and then declined off of IL-2. IL-2-induced Treg expressed Foxp3+ and were fully functional in *in vitro* suppression assays.
Similar immunologic and clinical responses have been observed in the ongoing phase II trial of low-dose rIL-2 for steroid-refractory chronic GVHD (DFCI 11-149; unpublished data). These data indicate that low-dose rIL-2 is safe in chronic GVHD and preferentially augments Treg. Similar Treg enhancement and clinical benefit was also documented in HCV-induced vasculitis [18]. Two studies of type 1 diabetes showed a similar increase in Tregs, in one there was worsening glucose metabolism but not in the other [20].

2. Rationale and Potential Benefits

Despite advances in immunosuppressive protocols, chronic allograft injury and loss remains a significant limitation in current clinical practice in transplantation. Tregs are capable of suppressing alloimmune responses and in many animal models have been shown to be highly effective in preventing graft injury. There are currently no therapies available to promote Tregs in vivo after transplant. Given the documented feasibility and safety of low-dose rIL-2 in HSCT recipients; including those with active steroid-refractory chronic GVHD, we propose that low-dose IL-2 may offer therapeutic benefit in organ transplantation. However, there are three limiting factors to the effective use of rIL-2 that

have been identified from these studies. Firstly drug toxicity; the use of *high dose* (as opposed to the ultra-low dose we are proposing) of rIL-2 has been associated with significant morbidities and even with low dose rIL-2 there appears to be a maximum tolerated dose, beyond which the side effects of rIL-2 are not well tolerated. Secondly the associated rise in the Treg population with low dose rIL-2 therapy is transient, and when rIL-2 is stopped, the size of the Treg population falls back towards baseline. Thirdly, as IL-2 is a growth factor for many different cell types, and although prior studies have not shown an effect on effector T cells at the dose we propose, IL-2 can increase populations of cells associated with the innate immune response, particularly NK cells, which may limit the efficacy of this approach and could increase the risk of intra-graft inflammation**.**

We posit that by using a low dose of rIL-2, we will target activation of high affinity IL2-receptors on Tregs while avoiding intermediate affinity IL-2 receptors expressed on innate immune cells and therefore avoiding activation of these cells which may be responsive to higher IL-2 levels. In this study we will be testing low dose rIL-2, based on studies by Koreth et al in GVHD, testing the safety of this approach and response of Treg populations in terms of expansion and *in vitro* function [19].

3. Objectives/Study Aims

a) To investigate the safety and tolerability of treatment with low dose rIL-2 in renal transplant recipients
b) To assess the immunologic impact of low dose rIL-2 in renal transplant recipients
c) To assess the efficacy of low dose rIL-2 in renal transplant recipients

4. Subject Selection

Patients will be recruited from BWH Renal Transplant clinic. Patients invited to enroll will be those taking stable doses of immunosuppression, unchanged for the prior month and who have undergone transplant kidney biopsy within the prior 3 months showing advanced interstitial fibrosis and tubular atrophy.

5. Inclusion criteria

1. Kidney transplant recipients,
2. >18 years and <75 years of age,
3. >6 months post Tx, on stable dose of immunosuppression
4. Transplant biopsy showing interstitial fibrosis and tubular atrophy of grade II or greater, with some evidence of lymphocytic infiltration
5. Ability to give informed consent

6. Exclusion criteria

1. Biopsy proven acute cellular rejection; greater than grade 1A
2. Baseline creatinine >3.5mg/dL
3. Patients with active infection, including Hepatitis B and C, HIV
4. Current or prior invasive malignancy
5. Patients who are pregnant or breastfeeding
6. Patients who are unable to give consent
7. Prior intolerance of/allergy to IL2

8. Inability to comply with treatment
9. History of thrombotic angiopathy including hemolytic uremic syndrome and thrombotic thrombocytopenic purpura
10. Symptomatic congestive cardiac failure or uncontrolled cardiac angina
11. Severe pulmonary disease
12. Active liver disease as defined by greater than 3x normal LFTs
13. Patients with known cirrhosis
14. Patients with ascites requiring large volume paracentesis more than twice in the past 6 months

7. Women of childbearing potential (WOCBP)

Women of childbearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of study drug.

WOCBP and male subjects with reproductive potential must agree to use a highly effective method of birth control during study treatment, and for 6 months after completion of treatment. Please refer to section 7.1 for acceptable methods of birth control.

7.1. Highly Effective Methods of Birth Control:

- Male condoms with spermicide
- Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena® by WOCBP subject or male subject's WOCBP partner
- Nonhormonal IUDs, such as ParaGard®
- Tubal ligation
- Vasectomy
- Complete Abstinence**

**Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.

8. Study Design and Duration

This will be an unblinded, pilot study of ultra-low dose daily sc IL-2 for eligible patients with evidence of allograft fibrosis and lymphocytic infiltration despite standard therapy. The dose used will be 1 $x10^6$ units/m^2/day, a dose previously shown to be safe, tolerable and associated with increased Tregs in a study of chronic graft versus host disease post bone marrow transplant. This dose is over an order of magnitude lower than those given for treatment of metastatic melanoma or renal cell carcinoma.

Five patients will be recruited at Brigham and Women's Hospital.

8.1. Stopping Rule

If two or more patients experience a complication or dose limiting toxicity, the study will be terminated early. Please refer to section 12 for Management of Toxicities.

8.2 Staggered Enrollment Plan/Duration

Each enrolled patient will be treated for 6 weeks and seen for study visits on week**s** 0, 1, 4, 6, 8, 10 and 16. Study recruitment will be staggered with the initial three patients being enrolled one month apart to minimize risk.

8.3 Early Termination Follow Up

If a patient is withdrawn, either voluntarily or by the PI, for any reason prior to study completion, they will be followed in clinic at one week and reviewed at four weeks from the time of their termination. We have Renal Transplant clinics on 4 days per week and patients will also be scheduled for review as needed according to any symptoms and adverse effects they may have noted. Laboratory and other investigations will be requested as clinically indicated. For grade 3 or greater toxicities, patients will be admitted to BWH under the care of the renal transplant service, as is clinically indicated. Patients who drop out prior to starting therapy with rIL-2 will be replaced.

8.4 Possibility of Extended Treatment

After completing 6 weeks of IL-2 therapy, patients who are experiencing benefit, defined as improved renal function from baseline level, and without significant side effects, will be have the option to continue IL-2 therapy for a further 6 weeks, at the discretion of the treating physician. Patients will be reassessed during this time with visits on weeks 8, 10, 12, 16 and 24.

9. Treatment Program

Treatment: Weeks 0-6, IL-2 $1x10^6$ units/m^2 SC qd

Study visits: Week 0

Week 1 (Day 7 ± 2 days)

Week 4 (Day 28 ± 3 days)

Week 6 (Day 42 ± 3 days)

Week 8 (Day 56 ± 3 days)

Week 10 (Day 70 ± 3 days)

Week 12 (Day 84 ± 3 days)*

Week 16 (Day 112 ± 3 days)

Week 24 (Day140 ± 3 days)*

*Only subjects in extended treatment phase will be seen on week 12 and week 20

Lab studies: To be drawn at all study visits. Please refer to the Study Procedures (Section 10) and the Schedule of Events (Section 16) for details.

10. Study Procedures

Patients will be fully assessed at study visit, including history, physical examination, symptom review and lab tests as outlined below. Vital signs (weight, BP, pulse, oxygen saturation) will be recorded. We will obtain clinical data for the duration of the study and for three months after completion from the electronic medical record (details below). Side effects and toxicity assessment will be recorded at visits and organ toxicities will be graded according to the NCI Common Toxicity Criteria. Please refer to Section 17 for the Schedule of Events.

10.1 Blood collection

Blood will be collected at each study visit (45mls per visit). We will test CBC with differential, CMP including Na, K, Cl, HCO3, BUN, Creatinine, eGFR, glucose, calcium, AST, ALT, alkaline phosphatase, bilirubin, albumin, protein and globulins at each visit.
LDH and immunosuppressive drug levels (i.e., Tacrolimus/cyclosporine/rapamycin) will be tested at visits on week 1 and 6, and at weeks 12 and 24 for those patients who continue study drug beyond week 6. Hypothyroidism was described in one patient treated with low dose IL-2 in a DFCI study, therefore we will check TSH at week 0 and week 6, and at weeks 12 and 24 for those patients who continue study drug beyond week 6. Three additional vials of blood, a total of 27 milliliters (8 ½ teaspoons), will be drawn for immunologic mechanistic assays including Treg assays, flow cytometry, cytokine analysis and anti-HLA antibody testing, at all study visits.

10.2 Urine collection

Patients will be asked to provide a urine sample in a sterile cup at each visit. Part of this sample will be analysed for urinalysis with sediment, urine creatinine and protein levels. Remaining urine will be stored in a -80^0 freezer for mechanistic studies.

10.3 Renal biopsy tissue

Excess material from the patient's pre-enrollment renal biopsy will be taken for analysis. If a renal biopsy is required for clinical care during the study time period, excess/discarded material will be taken for analysis. We will assess the biopsy for degree of rejection, type of infiltrating cells, PCR and RT-PCR

10.4 Mechanistic assay collection – Transplantation Research Center (TRC) labs

The BWH Transplantation Research Center (TRC) is a multifaceted group of clinicians and investigators, led by Director Anil Chandraker, MD, that strives to dramatically improve outcomes for recipients of transplanted organs. Formed in 2003 as a result of a generous gift from the Schuster family, the TRC is involved in basic, translational and clinical research. All mechanistic studies will be performed at the TRC.

Blood taken for mechanistic studies will be used for analysis of immune cell populations and their functions using flow cytometry, ELISPOT, PCR, RT-PCR, protein analysis and mRNA profiling. Cytokine and chemokine levels will also be analysed using ELISA and Luminex studies. A highly detailed form of flow cytometry, known as CyTOF, will be used to analyse immune cells. We will also assess for anti-HLA antibodies using Luminex studies. In these experiments, we will be focused on the effect of low dose rIL-2 treatment on Tregs but will also assess its effect on other immune cells to have a complete picture of its effect on transplant patients.
Urine stored for mechanistic studies will be analysed by PCR, ELISA, RT-PCR, mRNA profiling and protein analysis.
The stored specimens may be used for future assays to reevaluate biological responses as new tests are developed over time.
All biological samples will be stored with unique study IDs that are not labeled with any unique identifying information, such as name, MRN or birthdates.

10.5 Data to be collected

We will perform chart review and enter demographic information, clinical and laboratory data into a secure electronic database. Data for collection will include:

1. Demographics - age, race, and sex.
2. Transplant details – type of kidney donor, live vs. deceased, details of HLA antigen matching, early complications post transplant, presence of donor specific antibodies, prior rejection episodes and how those were treated,
3. Infection data – prior history of transplant related infections including BK virus, CMV, EBV, PCP and all infectious complications during the study period
4. Comorbid illness – details of all comorbidities and hospitalizations during the study period.
5. Laboratory data – renal function, hematologic, metabolic parameters and drug levels as outlined above; also details of prior renal biopsies and any new biopsies undertaken during the study period
6. Medications – all current medications the patient is taking during the study period

These data will be collected at enrollment and followed throughout the period the patient is enrolled until three months after the study has completed.

10.6 Drug treatment: rIL-2 1x 10^6 units/m^2

All patients will self-administer single daily subcutaneous injections of rIL-2. Patients will be trained to self-administer subcutaneous injections by Dr. Chandraker or Dr. McGrath. rIL-2 will be supplied free of charge by Prometheus Laboratories Inc. Proleukin is supplied as a lyophilized cake in single dose vial, containing 22x10^6 units, designed for IV administration. Pre-filled syringes will be prepared by pharmacy under sterile conditions, at the appropriate dose for each patient. These doses are stable for 14 days stored at 2-8^{0}C.

11. Risks and Discomforts

11.1. Potential drug side effects and toxicities of rIL-2

Much of the published literature on IL-2 relates to treatment with high dose IL-2, over an order of magnitude greater than the dose planned in this study. Many of the adverse events

related to high dose IL-2 have not been observed during studies of low dose treatment. Points 1-5 have been reported to varying degrees in patients treated with low dose IL-2. However, experience is limited and based on the potentially serious nature of these complications, we plan to advise patients of potential risk of the following adverse events and monitor them prospectively for the development of any of the following complications.

1. Constitutional symptoms: Fevers, malaise, fatigue, weakness
2. Infection: Transplant recipients are heavily immunosuppressed and infections are not uncommon in this population. Minor infections will not be considered a complication of IL-2 therapy. However, severe infections, including those requiring hospitalization, will be considered an adverse event and IL-2 will be stopped.
3. Thrombotic microangiopathy: Diagnosed as a combination of decreased platelet count, increased LDH and presence of schistocytes on peripheral blood smear
4. Renal – Acute renal failure. This is generally secondary to capillary leak syndrome or thrombotic microangiopathy. The occurrence of AKI will be managed as outlined below. High dose IL-2 is relatively contraindicated in patients with advanced renal impairment due to the risk of AKI. However to date, the group at DFCI has treated 64 patients in total with low dose IL-2 and have not observed renal toxicity (personal communication, John Koreth).
5. Hematologic – Leukopenia, thrombocytopenia, anemia
6. Renal transplant rejection – Due to the possibility of activation of other immune cells aside from Tregs
7. CNS – Malaise, confusion, somnolence.
8. Cardiovascular – Hypotension, supraventricular tachycardia, atrial fibrillation, peripheral edema
9. Capillary leak syndrome – High dose IL-2 has been associated with the development of hypotension, loss of vascular tone with edema, respiratory insufficiency, renal impairment and mental status changes. This has not been reported in series of patients treated with low dose IL-2.
10. Hypothyroidism – reported in one patient treated with low dose IL-2 at DFCI. We will monitor TSH during the study period as outlined above. If a patient develops hypothyroidism, IL-2 will be held to monitor for resolution. If required, levothyroxine replacement will be commenced.
11. GI – Nausea and vomiting
12. Dermatologic – Rash, itch

11.2. Pregnancy Risk

It is not known if this drug might cause problems if either the male or female is taking it at the time of conception or during pregnancy. Therefore, WOCBP and male subjects with reproductive potential must agree to use a highly effective method of birth control during study treatment, and for 6 months after completion of treatment. Please refer to section 7.1 for acceptable birth control methods.

11.3. Complications of procedures

Blood will be obtained by venipuncture at each visit. The risks of obtaining blood by venipuncture are generally limited to local bruising at the site of blood draw and rare transient dizziness. Blood drawing can contribute to the development of anemia, we will therefore withhold blood draws from patients under the following circumstances:

a) Active myocardial ischemia and hematocrit < 30%
b) Severe anemia (hematocrit <21%)

Collection of spontaneously voided urine is non-invasive and poses no risk to patients. All blood samples will be obtained by nursing staff or phlebotomists using standard precautions. We will not record sensitive personal health information.

There are minimal risks associated with subcutaneous injection of rIL-2. Injection site reactions such as pain, tenderness, bruising or injection site induration are generally mild. Patients will be trained in injection technique by Dr. Chandraker or Dr. McGrath and advised to rotate the sites of injection. Sharps bins will be provided for safe disposal of needles.

11.4. Psychosocial and non-medical risks

We will use study IDs rather than medical record numbers to label and track specimens and results. Medical Record Numbers will be linked to study IDs in a file that is located on a password-protected secure computer. We do not anticipate any excess psychosocial risks of study enrollment.

12. Management of Toxicities

Patients will be removed from the study and treatment with IL-2 will be discontinued immediately and not restarted, should any of the following circumstances arise:

- Anaphylaxis to IL-2
- Life threatening infection while on IL-2 therapy
- Other grade 4 toxic event
- AKI (defined as increase in creatinine to greater than 1.5 times the baseline level)
- Episode of acute allograft rejection
- Recurrent or unresolving grade 3 toxic event
- Severe pancytopenia (ANC < 500, Plts <10,000) not related to intercurrent infection

Patients who are withdrawn due to an adverse event will be assessed and followed at the Renal Transplant clinic as clinically indicated until their symptoms have completely resolved.

Lesser toxicities

In patients who experience a grade 2 event, IL-2 will be continued
If patients experience a grade 3 toxic event, IL-2 will be withheld. Treatment will be restarted if the symptoms resolve to grade 2 or less within one week. If grade 3 toxicity recurs, treatment with IL-2 will be abandoned.

13. Safety

13.1. Adverse Event (AE)

An AE is any *unfavorable* medical occurrence in a human subject enrolled in this study regardless of its casual relationship to study treatment. This may include:

- Abnormal sign (e.g. finding on physical exam or laboratory finding);
- Symptom;
- Disease.

13.2. Serious Adverse Event (SAE)

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

- Results in death;
- Is life threatening (places subject in immediate risk of death from the event as it occurred);
- Requires patient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect;
- Any clinically significant event that based on the judgment of the Investigator may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above.

13.3. Pregnancy

If a patient is found to be pregnant during the course of the study, treatment will be stopped immediately. The pregnancy will be reported to the Partners Human Research Committee via eIRB as an Other Event – Unanticipated Problem. The patient will be followed until the outcome of the pregnancy is known (spontaneous miscarriage, elective termination, normal birth or congenital abnormality), even if the patient was discontinued from the study. The pregnancy of a male patient's partner will similarly be reported to the IRB and followed. All reports of miscarriages and congenital abnormalities/birth defects will be considered SAEs and will be submitted for IRB, FDA and manufacturer review according to guidelines.

13.4. The following hospitalizations typically will not be considered SAEs in this study, but should be documented in the subject files as appropriate (ie. surgical procedure, AE log, etc.):

- A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event);
- Elective surgery, planned prior to signing consent
- Admissions as per protocol for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy);
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases;
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

13.5. Safety/data monitoring

All study subjects will be given physician-investigator contact information, so they can contact them for questions or concerns, twenty-four hours a day, 7 days a week. In

addition, each study subject will be given a wallet-sized Patient Safety Card which will briefly explain the study, some of the risks involved, and how to contact the study Principal Investigator. Please refer to section 20 for an example of the Patient Safety Card.

The PI is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care. All safety parameters, including laboratory assessments, will be reviewed by the PI at our weekly standing Clinical Research Working Group meeting, or before, if clinically significant.

13.6. Adverse Event Reporting to Partners Human Research Committee

All adverse events will be monitored and recorded on an ongoing basis in subject files. Adverse events which are unexpected and related or possibly related to the research and that indicate there is a new or increased risk to subjects will be reported via eIRB in Insight to the Partners Human Research Committee within 5 working days/7 calendar days of the date the investigator first becomes aware of the event. We will also follow manufacturer and FDA guidelines for adverse event reporting.Please see sections 13.7 and 13.8 for details.

13.7. IND Safety Reporting Requirements

Per 21 CFR 312.32(c)(1)(i), the Sponsor (Dr. Chandraker) will notify the FDA in an IND Safety Report when an event meets ALL of the following criteria:

- suspected adverse reaction (reasonable possibility of relationship to study drug)
- serious
- unexpected

Fatal or life-threatening events will be reported as soon as possible, but no later than 7 days of occurrence.
Serious and unexpected events will be reported as soon as possible, but no later than 15 days of occurrence.

13.8. Reporting to Prometheus Laboratories

A copy of any SAE report submitted to the Institutional IRB or FDA must be sent to Prometheus Laboratories. If Interleukin-2 is a suspect or co-suspect drug reported on the FDA Form 3500A MedWatch report, Prometheus Laboratories also requests a courtesy copy of the FDA Form 3500A MedWatch report that was submitted to the US Food and Drug Administration, via email or fax, to Drug Safety and Pharmacovigilance at Prometheus Laboratories, Inc. Please also include your contact information.

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

13.9. Data Safety Monitoring Board (DSMB)

Study Data including toxicities, AEs and SAEs will be reviewed at the quarterly DSMB meeting. Emergency meetings may also be convened should the need arise. Please refer to section 19 for the DSMB Charter.

14. Potential Benefits to subjects

This is primarily a pilot study to establish safety of rIL-2 in transplant recipients. We also hope to demonstrate efficacy in increasing Treg numbers in these patients. Patients enrolled will be those for whom no other treatment is currently available and are at high risk of progressive renal failure and graft loss. Our study is not designed to assess patient or transplant outcomes, but subjects may gain benefit if they develop increased numbers of circulating Tregs as this could help to improve the function or survival of their kidney transplant. Our hope is that this study will form the basis for a larger trial of longer duration to assess if such strategies to increase circulating Tregs may improve patient and transplant outcomes in the longer term.

15. Statistical Analysis

This is a small pilot study with five patients, designed to assess safety and tolerability of IL-2 treatment. Therefore our statistical analysis will be limited to description of changes in regulatory T cell and other immune cell populations with treatment with IL-2, including assessment of numbers, characteristics and function. We will also assess any symptoms, adverse events and outcomes experienced by the patients.

16. Privacy/medical information protection

All study-related computer files will be kept on a password-protected computer in a locked office. All study-related paper files will be kept in a locked office, in which only the IRB-approved study PI and Research Manager/Coordinator has access. The Institutional Review Board (IRB) at each institution, as well as relevant regulatory authorities, such as the FDA, may inspect the records at any time.

17. Schedule of Events (SOE)

Study Visits	Screening Wk 0	Visit WK 1 Day 7 ± 2 days	Visit WK 4 Day 28 ± 3 days	Visit WK 6[a] Day 42 ± 3 days	Visit WK 8 Day 56 ± 3 days	Visit WK 10 Day 70 ± 3 days	Visit WK 12[b] Day 84 ± 3 days	Visit WK 16 Day 112 ± 3 days	Visit WK 20[b] Day 140 ± 3 days
Procedures									
Informed Consent	X								
serum HCG or urine pregnancy test	X								
Medical History Full Physical Exam	X	X	X	X	X	X	X	X	X
Vitals: wt, BP, P, SpO_2	X	X	X	X	X	X	X	X	X
AE/SAE Assessment		X	X	X	X	X	X	X	X
Tac/CSA/Rapa troughs		X		X			X		X
Urinalysis w/sediment	X	X	X	X	X	X	X	X	X
Urine: Protein	X	X	X	X	X	X	X	X	X
Urine: Creatinine	X	X	X	X	X	X	X	X	X
CMP	X	X	X	X	X	X	X	X	X
CBC w/differential	X	X	X	X	X	X	X	X	X
LDH		X		X			X		X
TSH	X			X			X		X
TRC labs, mechanistics									
Urine (PCR, ELISA, RT-PCR, mRNA profiling and protein analysis)	X	X	X	X	X	X	X	X	X
Blood (flow cytometry, ELISPOT, PCR, RT-PCR, protein analysis, mRNA profiling, ELISA, Luminex, CyTOF)	X	X	X	X	X	X	X	X	X

[a]Decide if subject will continue treatment.
[b]Visit weeks 12 and 20 will occur only for subjects in extended treatment phase

18. References

1. Halloran PF. Immunosuppressive drugs for kidney transplantation. The New England journal of medicine 2004;351:2715-29.
2. Salama AD, Najafian N, Clarkson MR, Harmon WE, Sayegh MH. Regulatory CD25+ T cells in human kidney transplant recipients. Journal of the American Society of Nephrology : JASN 2003;14:1643-51.
3. Tsaur I, Gasser M, Aviles B, et al. Donor antigen-specific regulatory T-cell function affects outcome in kidney transplant recipients. Kidney international 2011;79:1005-12.
4. Zhang GY, Hu M, Wang YM, Alexander SI. Foxp3 as a marker of tolerance induction versus rejection. Current opinion in organ transplantation 2009;14:40-5.
5. Martinez-Llordella M, Lozano JJ, Puig-Pey I, et al. Using transcriptional profiling to develop a diagnostic test of operational tolerance in liver transplant recipients. The Journal of clinical investigation 2008;118:2845-57.
6. Akl A, Jones ND, Rogers N, et al. An investigation to assess the potential of CD25highCD4+ T cells to regulate responses to donor alloantigens in clinically stable renal transplant recipients. Transplant international : official journal of the European Society for Organ Transplantation 2008;21:65-73.
7. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nature immunology 2005;6:1142-51.
8. Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. Science 2006;311:1924-7.
9. Tang Q, Adams JY, Penaranda C, et al. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity 2008;28:687-97.
10. Webster KE, Walters S, Kohler RE, et al. In vivo expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. The Journal of experimental medicine 2009;206:751-60.
11. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. Jama 1994;271:907-13.
12. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1999;17:2105-16.
13. Aoyama A, Klarin D, Yamada Y, et al. Low-dose IL-2 for In vivo expansion of CD4+ and CD8+ regulatory T cells in nonhuman primates. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2012;12:2532-7.
14. Koulmanda M, Qipo A, Fan Z, et al. Prolonged survival of allogeneic islets in cynomolgus monkeys after short-term triple therapy. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2012;12:1296-302.
15. Long S, Buckner J, Greenbaum C. IL-2 therapy in type 1 diabetes: "Trials" and tribulations. Clinical immunology (Orlando, Fla) 2013.
16. Long S, Rieck M, Sanda S, et al. Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs β-cell function. Diabetes 2012;61:2340-8.
17. Millington T, Koulmanda M, Ng C, et al. Effects of an agonist interleukin-2/Fc fusion protein, a mutant antagonist interleukin-15/Fc fusion protein, and sirolimus on cardiac allograft survival in non-human primates. J Heart Lung Transplant 2012;31:427-35.

18. Saadoun D, Rosenzwajg M, Joly F, et al. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. The New England journal of medicine 2011;365:2067-77.
19. Koreth J, Matsuoka K, Kim HT, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. The New England journal of medicine 2011;365:2055-66.
20. Hartemann A, Bensimon G, Payan CA, et al. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. Lancet Diabetes 2013.

19. DSMB Charter

Prinicipal Investigator: Anil Chandraker, MD
Brigham and Women's Hospital

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to the Principal Investigator of this Investigator-Initiated study, to monitor participant safety, data quality and evaluate the progress of the study.

Membership
DSMB Chair: Andrew Siedlecki, MD
2 other members of DSMB: Jean Francis, MD (Boston University) and Paulo Martins, MD PhD (UMass)

DSMB Responsibilities
The DSMB responsibilities are to:

- Evaluate the progress of the trial, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- Review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigator;
- Protect the safety of the study participants;
- Make recommendations to the Principal Investigator, and, if required, to the Food and Drug Administration (FDA) concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study.

The DSMB will discharge itself from its duties when the study is complete.

Board Process
At each meeting, the DSMB will discuss the protocol, suggested modifications, and establish guidelines to study monitoring by the Board. The a designated DSMB Chairperson, in consultation with the Principal Investigator and study staff, will prepare the agenda to address the review of study materials, and the reporting of adverse events.

Meetings of the DSMB will take place quarterly, until the completion of the study. An emergency meeting of the DSMB may be called at any time should participant safety questions or other unanticipated problems arise.

Meetings shall be closed to the public because discussions may address confidential participant data. Meetings are attended by the Principal Investigator and members of his/her staff. Meetings may be convened as conference calls as well as in-person.

Meeting Format
DSMB meetings will consist of open and closed sessions. Discussion held in all sessions is confidential. The Principal Investigator and key members of the study team attend the open

sessions. Open session discussion will focus on the conduct and progress of the study, including participant accrual, protocol compliance, and problems encountered.

The closed session will be attended by the DSMB members.

Each meeting must include a recommendation to continue or to terminate the study made by a formal DSMB majority or unanimous vote. Should the DSMB decide to issue a termination recommendation, the full vote of the DSMB is required. In the event of a split vote, majority vote will rule and a minority report should be appended. The DSMB Chair provides the tiebreaking vote in the event of a 50-50 split vote.

A recommendation to terminate the study may be made by the DSMB at any time by majority vote. In the event that the DSMB requires the study to be terminated, the PI will be immediately informed about their decision.

Meeting Materials
DSMB interim report templates will be prepared by the study staff, to be reviewed by the DSMB members at the first meeting. Interim data reports generally consist of two parts:

Part 1 - Open Session Report and
Part 2 - Closed Session Report

Format and content of the reports for both the open and closed sessions and plans for interim analyses should be finalized and approved at the initial DSMB meeting, although changes throughout the trial may be requested by the Board.

The reports will list and summarize safety data and describe the status of the study. All meeting materials should be sent to the DSMB members at least *1 business day* prior to the meeting.

Part 1 - Open Session Reports: Open session reports generally include administrative reports that describe participants screened, enrolled, completed, and discontinued, as well as baseline characteristics of the study population. Other general information on study status may also be presented. Listings of adverse events and serious adverse events as well as any other information requested by the DSMB may also be in the open session report. The DSMB may direct additions and other modifications to the reports on a one-time or continuing basis.

Part 2 – Closed Session: The closed session will be for DSMB members only to discuss what was presented in the Open Session, and any concerns or queries they may have.

Reports from the DSMB
A formal report containing the recommendations for continuation or modifications of the study will be prepared by the DSMB Chairperson. Once approved by the DSMB members, the DSMB Chair will forward the formal DSMB recommendation to the Principal Investigator, within 2 weeks of the DSMB meeting. It is the responsibility of the Principal Investigator to distribute the DSMB recommendation to all co-investigators and to ensure that copies are submitted to all the IRBs associated with the study.

Confidentiality
All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

20. Example of Patient Safety Card (will be wallet sized)

Front:

PATIENT SAFETY CARD – PLEASE KEEP IN YOUR WALLET

This patient is in a clinical research study. Treatment in this very short term study is with **LOW DOSE,** daily, subcutaneous Proleukin® (recombinant IL-2), manufactured by Prometheus Laboratories.

Patient Name:_____ DOB:_____
Address: _____Phone:_____
Emergency Contact (1):_____Phone:_____
Emergency Contact (2):_____Phone:_____

_____

Kidney Transplant Patient – Brigham and Women's Hospital (BWH)

Back:

PATIENT SAFETY CARD – PLEASE KEEP IN YOUR WALLET

Proleukin® Dose: $1x10^6$ units/m^2 SC qd (duration 6-12 weeks)
Package Insert is on file at BWH IDS (617) 732-6410
Pager Operator: 617-732-6660 BWH IDS Pager #33633

Anil Chandraker, MD is the doctor in charge of this research study at BWH. You can call him at 617-732-7412 Monday – Friday 9-5.

For **urgent concerns**, you can call: Subject #:_____
BWH pager operator: 617-732-6660 IND #_____
(24 hours a day, 7 days a week) NCT#_____
Please ask to have Dr. Chandraker paged at #34197 IRB # _____