STATUS PAGE
PROTOCOL **14-479**

Closed To New Accrual

Closure Effective Date: 04/28/2017
Reason: Study Accrual Goal Met

No new subjects may be enrolled in the study- as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

DFCI Protocol No: 14-479

Front Sheet

Report Generated: 06/12/2018 09:41 AM

Title: A Phase II Trial of Low-Dose Interleukin-2 (IL-2) Added to Extra-Corporeal Photopheresis for Steroid-Refractory Chronic Graft-Versus-Host-Disease

Overall Institution: Dana-Farber Cancer Institute

Overall Principal Investigator	Email	Institution
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Sponsor Name	Sponsor Protocol No	Roles	Grant Number(s)
DF/HCC Investigator		Funding; Regulatory	
Prometheus Laboratories		Funding	
Dana-Farber/Harvard Cancer Center		Funding	

Total Study-Wide Enrollment Goal: 25 **Total DF/HCC Estimated Enrollment Goal:** 25

Phase: II

Age: Adults

Age Ranges: 18+

Will all subjects be recruited from pediatric clinics?

CTEP Study: No

Management Group(s): DF/HCC Transplant
DFCI/BWH Transplant
DFCI/BWH Treatment Plan (BMT/Non-Research)

Primary Management Group: DF/HCC Transplant

Investigational Drug? This study does not use a Drug/Biologic.

Investigational Device? This study does not use an Investigational Device.

IRB of Record:

Risk Category: Greater Than Minimal Risk

Protocol Involves: Human Material Banking; Human Material Collection; Immunotherapy; Questionnaires/Surveys/Interviews

Date Range: (Medical Record Review and Specimen Collection studies)

Participating Sites under the DFCI IRB

Institution: Brigham and Women's Hospital
Dana-Farber Cancer Institute

Participating Institutions Under Other IRB

None

Protocol Number: 14-479

Approval Date: 12/02/2014 (IRB meeting date when protocol/consent approved or conditionally approved)

Activation Date: 02/02/2015 (Date when protocol open to patient entry)

Approval signatures are on file in the Office for Human Research Studies, tel. 617-632-3029.

Date Posted	Revised Sections	IRB Approval Date	OHRS Version Date
[redacted]	[redacted]	[redacted]	[redacted]
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Protocol Version Date: Version 2.0; August 15, 2016

NCI Protocol #*:* N/A

Local Protocol #: 14-479

TITLE: A PHASE II TRIAL OF LOW-DOSE INTERLEUKIN-2 (IL-2) ADDED TO EXTRA-CORPOREAL PHOTOPHERESIS FOR STEROID-REFRACTORY CHRONIC GRAFT-VERSUS-HOST-DISEASE

Principal Investigators:
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Responsible Data Manager: N/A

Agent(s): Interleukin-2 (Proleukin®) (exempt) – Prometheus Laboratories, Inc

SCHEMA

OBJECTIVES

To assess the efficacy of daily subcutaneous (SC) low-dose interleukin-2 (IL-2) added to extra-corporeal photopheresis in patients with chronic GVHD (cGVHD)

PATIENT POPULATION

- Patients with cGVHD requiring systemic therapy. Patients with either extensive cGVHD or limited cGVHD requiring systemic therapy are eligible.
- Inadequate response to at least 4 weeks of prednisone at a dose of ≥0.25 mg/kg/day (or equivalent).

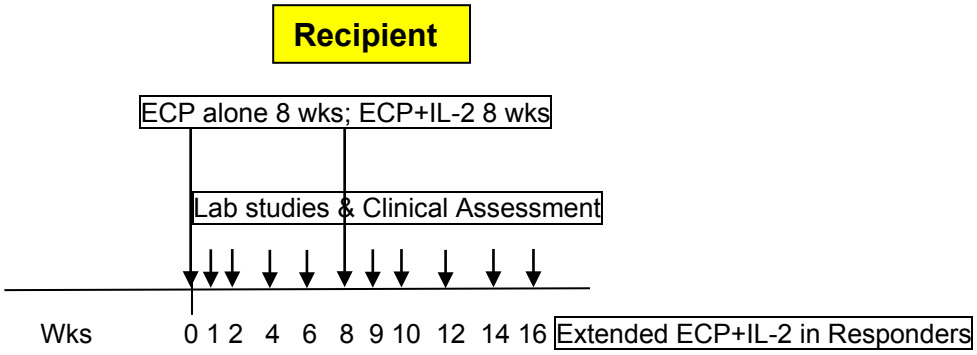
Specific inclusion and exclusion criteria are detailed in section 3.1 and 3.2 respectively.

NUMBER OF PATIENTS

25

STUDY DESIGN AND METHODOLOGY

Phase II



SAFETY AND EFFICACY DATA

The following assessments will be conducted to assess the efficacy of ECP plus IL-2:

- Overall cGVHD response rate at week 16
- Toxicity of 8 week daily IL-2 added to ECP
- Immunologic effects of ECP plus low-dose IL-2
- Prednisone use during ECP plus low-dose IL-2
- Overall and progression-free survival, non-relapse mortality, and relapse at 1 year after study entry

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1. OBJECTIVES

1.1 Study Design

- Phase II trial to assess the efficacy of 16 week extracorporeal photopheresis (ECP) with the addition of daily low-dose subcutaneous (SC) interleukin-2 (IL-2) (Proleukin®) for weeks 8-16 in patients with steroid-refractory chronic graft-versus-host disease (cGVHD).

1.2 Primary Objective

- To determine the overall clinical response rate of ECP plus low-dose daily SC IL-2 in steroid-refractory cGVHD.

1.3 Secondary Objectives

- **To determine toxicity of ECP plus low-dose SC IL-2 therapy.**
- To assess the immunologic effects of ECP plus low-dose daily SC IL-2
- **To determine ongoing prednisone use with ECP plus low-dose IL-2 therapy.**
- To assess overall survival, progression-free survival, non-relapse mortality and relapse at 1 year after start of ECP plus low-dose IL-2.

2. BACKGROUND

2.1 Study Disease: Graft vs. Host Disease

For many patients with severe benign and malignant hematologic disorders, allogeneic hematopoietic stem cell transplant (HSCT) offers the only opportunity for cure. Unfortunately, significant obstacles remain: most notably disease recurrence and GVHD. Over 40% of adult patients undergoing HSCT relapse while more than 50% will develop chronic GVHD (cGVHD), a debilitating condition with multi-system immune manifestations associated with a considerable morbidity and mortality. [1,2] Although the incidence in the pediatric population is lower, cGVHD remains a leading cause of non-relapse morbidity and mortality following allogeneic HSCT for malignant disease, occurring in 20 to 50% of children surviving greater than 100 days post-HSCT. [3] Donor cell mediated immune responses are responsible for GVL and GVHD reactions. Inadequate recognition and destruction of residual tumor cells by a newly engrafted donor immune system permits recurrence of a patient's malignancy, while uncontrolled reactions against host antigens lead to GVHD. [4,5] Chronic GVHD pathogenesis involves inflammatory T- and B-cell responses to allogeneic (donor/recipient polymorphic) and autologous (donor/recipient non-polymorphic) antigens.

cGVHD remains a common problem and major therapeutic challenge after allogeneic HSCT, and long-term survivors often experience impaired quality of life and increased late mortality. [6] The increasing use of mobilized peripheral blood progenitor cells rather than bone marrow as a source of stem cells for HCT has resulted in a clear increase in the incidence of cGVHD. [7,8] The inflammatory or fibrotic changes associated with cGVHD most commonly involve the skin, eyes, mouth, liver and respiratory tract. Systemic steroids are routinely used as first-line therapy to treat cGVHD but have limited efficacy and considerable toxicity. Second-line treatment options are limited and steroid-refractory cGVHD presents a major therapeutic challenge.

Extracorporeal photopheresis (ECP) is an apheresis procedure that consists of UVA irradiation of apheresis-collected, autologous leukocytes that are sensitized with 8-methoxypsoralen (8-MOP) and subsequently reinfused. The mechanisms of ECP are incompletely understood. Most data about the mechanism of ECP are derived from mouse models, which support the regulatory T-cell (Treg) hypothesis[9-11]. Treg are naturally occurring CD4+CD25+FOXP3+ T lymphocytes that comprise ~5-10% of the circulating CD4+ T cell population and act to dominantly suppress autoreactive lymphocytes and control innate and adaptive immune responses. [12-19] Additionally, in the context of ECP, induction of immature and plasmacytoid dendritic cells (DCs) through apoptotic T cells may precede increases in T_{reg}[20-23]. Human data on ECP mechanisms selectively report T_{reg}[24,25] or DC phenotype[26], but in heterogeneous populations, often without inclusion criteria or pre-ECP baseline data.

Despite mechanistic ambiguity, ECP is a routinely recommended and CMS-approved second-line treatment for cGVHD.[27] However, while safe, clinical benefit of ECP is limited. Approximately half of treated patients do not have a clinical response; partial response is the norm for responders; and taper of immune suppressants during extended ECP is slow and often incomplete. Improvements in treatment efficacy that can make ECP a more efficient therapy, with responses and steroid tapers achieved more quickly, is desirable.

2.2 Study Agent: Interleukin-2

Native interleukin-2 (IL-2), a secreted glycoprotein with a molecular weight of approximately 15 kilodalton (kD), was first identified in 1976 as a growth factor for T lymphocytes. It is produced by the majority of human CD4+ lymphocytes; and some cells of the CD8+ T-cell phenotype. IL-2 is known to play a central role in the generation of immune responses.

In cancer clinical trials, high-dose recombinant IL-2 (e.g. IV bolus dose of 600,000 international units (IU)/kg every 8 hours for up to 14 doses) demonstrated antitumor activity in metastatic renal cell carcinoma (RCC) and metastatic melanoma. IL-2 was approved for the treatment of metastatic RCC in Europe in 1989 and in the US in 1992. In 1998, approval was obtained to treat patients with metastatic melanoma. Recombinant human IL-2 (Aldesleukin) (Proleukin®-Novartis Inc. & Prometheus Labs Inc.) is currently approved by the United States Food and Drug Administration (US FDA).

2.2.1 Mechanism of Action

Native interleukin-2 (IL-2) was initially identified as a lymphocyte growth factor, and thought primarily to promote effector T cell responses in vivo, is now known to be a cytokine critical for the development, expansion, survival and peripheral activity of regulatory T cells (Treg). [28,29]

Treg are naturally occurring CD4+CD25+FOXP3+ T lymphocytes that comprise ~5-10% of the circulating CD4+ T cell population, act to dominantly suppress autoreactive lymphocytes, and control innate and adaptive immune responses. [12-19] Treg may be of benefit in the graft-versus-host disease (GVHD) context. In a murine model, a 1:1 mix of CD4+CD25+ Treg and CD25- effector T cells added to bone marrow stem cells suppressed alloimmune activation and GVHD without increasing malignant relapse post-transplant. [30] In the clinical context, we documented impaired Treg reconstitution in allogeneic transplant recipients with active chronic GVHD (cGVHD). [31,32]

This hitherto unexpected role for IL-2 in Treg homeostasis and function may explain its limited efficacy as anti-cancer therapy, and explain in part the finding that in-vivo administration of IL-2 plus syngeneic T cell depleted donor marrow prevents GVHD after mismatched murine allo-

SCT, without impacting GVL responses. [33,34] More directly, in murine allo-HSCT models co-infusion of Tregs expanded ex-vivo with IL-2 also resulted in suppression of GVHD, with improved immune reconstitution and preserved GVL responses. [35,36] In humans, IL-2 has been shown to regulate FOXP3 expression in Tregs and induce their expansion in-vivo. [37] We hypothesized that in patients with active cGVHD, Treg enhancement in-vivo with IL-2, if achievable, may help suppress effector T cell responses to allo-antigens (and auto-antigens) expressed in the recipient, and promote tolerance to these antigens.

2.2.1.1 Nonclinical Pharmacology

Il-2 pharmacokinetics has been evaluated in mice, rats, rabbits, sheep, pigs, cynomolgus monkeys. All species tested exhibited effects that were directly or secondarily related to the pharmacological actions of IL-2. After SC administration, absorption of IL-2 was slow and the mean resident time was prolonged compared with IV administration. In general, the pharmacokinetics of IL-2 appear to be linear across tested species (including humans), and are discussed in the clinical pharmacology section below.

2.2.1.2 Nonclinical Toxicity

IL-2 administration by the SC route has been evaluated in several multiple-dose studies in rats for up to 13 weeks of daily administration (Study Numbers 452480, 452496, and CIQ/002). They confirmed that the SC high-dose toxicity profile in rats was comparable to the high-dose toxicity profile after IV administration. IL-2-related findings included lymphocytosis, eosinophilia, slight anemia, extramedullary hematopoiesis, lymphoid hyperplasia, hepatomegaly and splenomegaly. Infiltrative and proliferative changes were seen in many organs, including liver, lung, lymph nodes, kidney, spleen and bone marrow. In rabbit SC tolerability studies (Study Numbers 562794 and 3391.9), after 7 days of dosing, local inflammatory responses at the injection site were consistent with the known effects of IL-2 and with injection-site reactions reported in human trials.

In summary, the toxicity of IL-2 in animals has been shown to be dose- and duration-related, with all toxic effects being directly or secondarily related to IL-2 pharmacological activity. In all species, treatment-related effects were fully or partially reversible after a treatment-free period of 2-4 weeks. Findings were comparable after repeated IV or SC administration of IL-2. The severity of target organ toxicity has been correlated with the extent of inflammatory cell infiltration into these organs. Biological effects in animals were generally similar to effects reported in clinical trials. However, these MTD studies relate to the high-dose IL-2 used for treatment of metastatic RCC and melanoma, rather than the low-dose IL-2 proposed for in-vivo

Treg enhancement in our study. Additional information regarding the nonclinical pharmacology and toxicology of IL-2 may be found in the September 10, 2003 Proleukin Investigator's Brochure (Chiron Corporation).

2.2.1.3 Clinical Pharmacokinetics and Pharmacodynamics

IL-2 pharmacokinetics has been evaluated in patients with cancer and with HIV. Following a short IV infusion, the pharmacokinetic profile of IL-2 was characterized by high plasma concentrations, rapid distribution into the extravascular space, and elimination from the body with a half-life of 1-2 hours. After SC administration absorption of IL-2 was slow and the mean resident time was prolonged compared with IV administration. Maximum plasma concentration of IL-2 in HIV patients was achieved within 2-5 hours after SC dosing and the terminal phase half-life was estimated at 5 hours, suggesting prolonged absorption.

The clearance rates for bolus infusion and continuous IV infusions are approximately equal. As expected from its short half-life, steady state is expected to be reached approximately 6 hours after infusion. Data on SC IL-2 administration suggest that approximately 35% is absorbed into the bloodstream, with greater bioavailablity (>60%) reported in a study involving HIV-infected participants.

Five days of treatment by either SC or continuous IV administration in cancer patients or HIV patients resulted in a time-dependent increase in IL-2 clearance. This increase was associated with an increase in serum levels of soluble IL-2 receptor. Drug-free days between cycles of IL-2 dosing restored the clearance of IL-2 to its initial value.

There are at least 3 mechanisms of clearance that account for the systemic removal of IL-2: glomerular filtration, peritubular extraction, and an inducible receptor-mediated mechanism (in man). Peritubular extraction of small peptides and proteins from the postglomerular capillaries into the renal tubules and subsequent intracellular catabolism is a renal mechanism of elimination that occurs independent of glomerular filtration. Receptor-mediated clearance is primarily a function of IL-2 engaging its specific cellular receptor on responsive cells. The majority of IL-2 receptors are on T cells, and NK cells, though other cell types such as B cells have functional IL-2 receptors.

In patients with metastatic melanoma or renal cell cancer treated with high-dose bolus IL-2, nearly 6 fold increase in CD4+CD25+ Treg cells was observed, with 4 fold increase in the frequency of circulating CD4+FOXP3+ Treg cells. [38] Considerable clinical data on the effects of IL-2 in patients with metastatic cancer have been accumulated over the past two decades. At the high doses of IL-2 used in most cancer trials, considerable toxicity has been documented, with only occasional tumor responses. Fever, hypotension, jaundice, and azotemia have been frequent complications, often necessitating admission to intensive care units. HSCT patients are unlikely to tolerate the toxicity of high-dose IL-2.

Low-dose IL-2, administered S.C. for extended periods, has been evaluated in patients with HIV infection and cancer. In one study of 'ultra-low-dose' IL-2, seven patients with HIV and Non-Hodgkin's Lymphoma in first remission received 1 x 10^6 IU/m^2/d of IL-2 (Chiron). [39]. After ~8 weeks of treatment, single-agent IL-2 therapy led to statistically significant, proportional increases in NK cells (1.6-fold) and Tregs (9-fold). Other lymphocyte subsets were not significantly changed. Toxicity was mild (fatigue, local pruritis, myalgia, increased transaminases etc.), with no Grade 3 adverse events.

Low-dose IL-2 has also been safely used in patients with hematologic malignancy after allogeneic HSCT. In one study, IL-2 (Amgen, Roche) was administered by continuous IV infusion to 29 asymptomatic patients after CD6+ T-cell depleted (TCD) allogeneic HSCT at doses ranging from 2-6 x 10^5 IU/m^2/d, (≈ 0.6-1.8 x 10^6 U/m^2/d of IL-2 (Chiron): personal communication) for periods of up to 3 months. [40] Low dose IL-2 was well tolerated, with only 4 patients withdrawn early due to toxicity. Interestingly, acute GVHD developed in only 1 of the 29 patients. In addition to the expected NK cell expansion, in 7 of 8 patients evaluated, a 45% median increase in CD4+ CD25+ T lymphocytes occurred, likely representing Tregs. Further, a median ~8.5 fold increase of FOXP3 expression was noted, also indicating substantial Treg enhancement. [37]

Since low-dose IL-2 has been tolerable post allo-SCT, and can result in significant expansion of Tregs that function to suppress allo-immune responses underlying GVHD, we postulated that it may be safe and effective therapy for active chronic GVHD. Since IL-2 also has stimulatory effects on cytotoxic T and NK cells, it was however possible that exacerbation or progression of chronic GVHD may occur while on IL-2.

2.2.1.4 Clinical Experience

In DFCI 07-083, we assessed safety of low-dose interleukin-2 in participants with refractory active cGVHD. [41] The primary objective was to determine the MTD and toxicity of an 8-week course of low-dose IL-2 in chronic GVHD participants with an inadequate response to steroids. Eligibility included cGVHD that had not responded to at least 0.25 mg/kg prednisone for a 4 week period, the absence of infection, and stable doses of immune suppression for 4 weeks prior. The study had a Phase 1 dose escalation design with 3 dose levels (0.3-, 1-, 3-; x 10^6 IU/m^2/day for 8 weeks).

29 participants accrued: 28 evaluable for toxicity; 23 for response. IL-2 at 1 x 10^6 IU/m^2/day was determined to be MTD. Two participants developed dose-limiting-toxicity (thrombotic microangiopathy). None experienced GVHD flare. There was no malignant disease relapse. 12 of 23 participants had objective clinical responses. Low-dose IL-2 selectively increased Treg counts in-vivo (Figure 1) without impacting conventional CD4+ T (Tcon) counts. The Treg:Tcon ratio also rose (Figure 2). NK cell counts rose to a lesser extent. Low-dose IL-2 did not impact CD8+ T, B, or NKT counts. Treg count and Treg:Tcon ratio remained elevated at 8 weeks of IL-

2, then declined off IL-2. IL-2-induced Treg expressed FoxP3+ and were functional in Tcon suppression assays. Importantly, clinical and immunologic responses were sustained in responders on extended-duration IL-2 therapy beyond 12 weeks, enabling a taper of concomitant immunosuppression.

Figure 1 (below left). Expansion of CD4 Treg during treatment with daily low-dose IL-2. Gating on CD3+CD4+ T, Treg are defined as CD25+CD127- (black) and Tcon are CD25-CD127+ (blue). The %Treg within the CD4 gate is indicated in each plot. Figure 1 depicts a representative patient on Dose Level B.

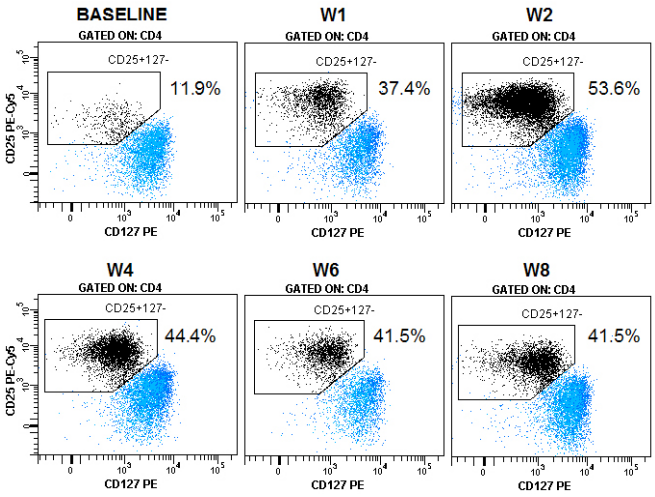

Figure 1

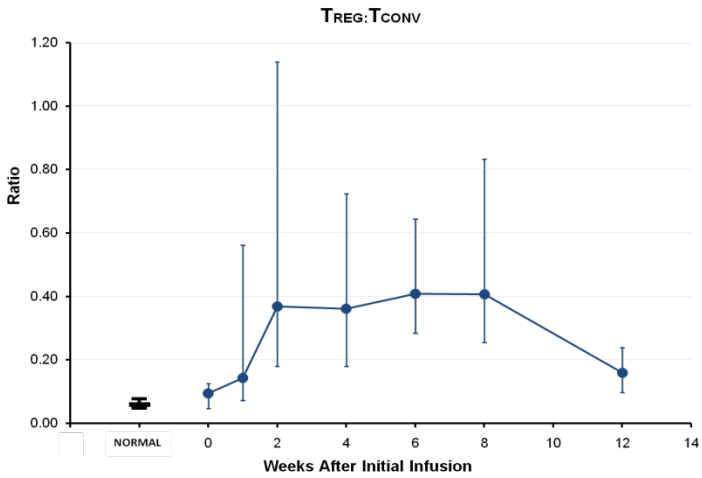

Figure 2

Figure 2 (above right): Median Treg:Tcon ratio on IL-2 therapy. There was a rapid rise and sustained plateau within a few weeks of starting IL-2, and a decline after stopping IL-2. Medians with inter-quartile range are shown.

These data indicate that low-dose IL-2 is safe in cGVHD and preferentially augments Treg. Similar Treg enhancement and clinical benefit was also documented in HCV-induced vasculitis.[42] However, despite Treg enhancement, clinical responses occurred in only half the treated cGVHD participants; that we have confirmed in our recently accrued phase II trial (DFCI 11-149, unpublished data). Additional combination-immunomodulatory strategies to enhance IL-2-mediated immunologic and clinical responses may be beneficial.

2.3 Rationale

The most common indication for ECP is glucocorticoid-refractory cGVHD after allogeneic HSCT. Initial studies in mouse models of acute GVHD indicate that the therapeutic mechanism of ECP is dependent on $CD4^+CD25^+FOXP3^+$ regulatory T cells (T_{reg}), which act to control auto- and alloimmune responses mediated by conventional T cells (T_{con}). However, human data in support of the ECP T_{reg} hypothesis are scant. In

parallel, human trials of low-dose interleukin-2 (IL-2) for glucocorticoid-refractory cGVHD show clinical responses alongside in vivo increases in T_{reg} number and function. As both ECP and low-dose IL-2 likely work through in vivo T_{reg} enhancement, there is significant biologic plausibility that ECP treatment can be augmented with low-dose IL-2.

Combination ECP and low-dose IL-2 is a novel and innovative strategy that offers conceptual and practical advantages over either approach alone. The combined intervention strategy is designed to maximally modulate T_{reg} expansion in order to better suppress cGVHD. IL-2 delivers a proliferative and survival signal to T_{reg} both in vitro and in vivo. We previously showed that low-dose IL-2 can be safely administered to cGVHD patients and preferentially expands T_{reg} in vivo to induce clinical responses. The one randomized, phase II trial of ECP for cGVHD showed that complete or partial skin responses were higher in the ECP group (40% vs. 10%, P=0.002)[43]. Of all GVHD manifestations, skin GVHD is the most responsive to ECP.[44] IL-2 therapy likewise has greatest success with skin responses (44% response rate)[41]. **To maximally enhance suppressive T_{reg} for cGVHD control, we will evaluate the mechanism and efficacy of ECP plus the sequential addition of daily low-dose IL-2 administration in patients with glucocorticoid-refractory cGVHD.**

Importantly, 4 DFCI patients are already receiving IL-2+ECP for refractory cutaneous cGVHD with no side effects. All had objective cGVHD responses at 3 months with increased skin suppleness and all elected to continue ECP+IL-2. Importantly, 2 of 2 subjects tested had an in vivo Treg rise with ECP+IL-2 compared to monotherapy. One patient with a PR to IL-2 initiated ECP during extended IL-2 therapy, with enhanced cGVHD clinical response and a 1.7-fold rise in Treg compared to IL-2 alone. In another patient with inadequate response to ECP, the addition of low-dose IL-2 induced cGVHD response along with a 33-fold rise in Treg compared to ECP alone.

We therefore plan a phase II clinical trial combining ECP plus low-dose IL-2 for glucocorticoid-refractory cGVHD. Our **short-term goals** are to evaluate the T_{reg} impact of ECP and of ECP plus low-dose IL2 in glucocorticoid-refractory cGVHD. *We hypothesize that clinical and immunologic responses to ECP can be further enhanced by low-dose IL-2; as both ECP and IL-2 quantitatively and qualitatively enhance T_{reg} function in cGVHD.* We will evaluate these hypotheses in a 16 week trial of twice-weekly ECP in weeks 1-16, with the sequential addition of daily low-dose IL-2 dosed at $1x10^6$ IU/m^2/day during weeks 8-16, with the **long-term goal** of optimizing ECP treatment for cGVHD.

2.4 Correlative Studies Background

As in the previous Phase I clinical trial, peripheral blood samples obtained before, during and after ECP and ECP + IL-2 therapy will be used to assess the immunologic effects of treatment. The types of studies carried are briefly summarized below.

Phenotypic analysis of lymphocyte subsets: Incubation of peripheral blood samples with monoclonal antibodies specific for lymphocyte markers is used routinely to identify functionally distinct lymphocyte subsets. After incubation of peripheral blood cells with directly fluorochrome-conjugated monoclonal antibodies, individual subsets are enumerated by flow cytometry. In this clinical trial, antibody panels have been developed to identify CD4+ regulatory T cells as well as other CD4 and CD8 T cell subsets, B cells, dendritic cell (DC) subsets, and natural killer (NK) cells. These studies will allow us to measure quantitative changes of individual populations that occur as a result of IL-2 treatment. We will correlate these changes with dose of ECP treated cells, determined by flow cytometric cell counts of the reinfused buffy coat from each ECP procedure.

Plasma cytokines: ELISA assays will be used to measure levels of IL-2 in plasma samples. Other cytokines, such as IL-7, IL-10, and IL-15, which play a role in T cell homeostasis, can also be measured in these samples.

Functional assays: To assess the functional capacity of regulatory T cells that expand in response to ECP and ECP + IL-2 treatment, selected samples will be used to assess the immune suppressive function of these cells. In these experiments, Treg are purified by high speed cell sorting and subsequently tested for their ability to suppress the proliferation of autologous T cells.

Taken together, these assays will define and quantify the immunologic effects of ECP and ECP + IL-2 treatment on regulatory T cells and other immune cells.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Recipients of 7-8/8 HLA matched adult donor allogeneic stem cell transplantation with myeloablative or non-myeloablative conditioning regimens.

3.1.2 Participants must have steroid-refractory cGVHD. Steroid-refractory cGVHD is defined as having persistent signs and symptoms of cGVHD (Appendix D; section 17.4) despite the use of prednisone at ≥ 0.25 mg/kg/day (or 0.5 mg/kg every other day) for at least 4 weeks (or equivalent dosing of alternate corticosteroids) without complete resolution of signs and symptoms. Patients with either extensive chronic GVHD or limited chronic GVHD requiring systemic therapy are eligible.

3.1.3 Stable dose of corticosteroids for 4 weeks prior to enrollment. Exception permitted with overall PI approval.

3.1.4 No addition or subtraction of other immunosuppressive medications (e.g., calcineurin-inhibitors, sirolimus, mycophenolate-mofetil) for 4 weeks prior to enrollment. The dose of immunosuppressive medicines may be adjusted based on the therapeutic range of that drug

3.1.5 Patient age ≥18 years old. Because no dosing or adverse event data are currently available on the use of IL-2 in participants <18 years of age, children are excluded from this study.

3.1.6 Estimated life expectancy greater than 3 months.

3.1.7 ECOG performance status 0-2 (Appendix A; section 17.1).

3.1.8 Participants must have adequate organ function as defined below:

- Hepatic: Adequate hepatic function (total bilirubin <2.0 mg/dl-exception permitted in patients with Gilbert's Syndrome; AST (SGOT)/ALT (SGPT) ≤2x ULN), unless hepatic dysfunction is a manifestation of presumed cGVHD. For patients with abnormal LFTs as the sole manifestation of cGVHD, documented GVHD on liver biopsy will be required prior to enrollment. Abnormal LFTs in the context of active cGVHD involving other organ systems may also be permitted if

the treating physician documents the abnormal LFTs as being consistent with hepatic cGVHD, and a liver biopsy will not be mandated in this situation.

- Renal: Serum creatinine within normal institutional limits or creatinine clearance ≥ 60 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal.
- Pulmonary: FEV1 ≥ 50% or DLCO(Hb) ≥ 40% of predicted, unless pulmonary dysfunction is deemed to be due to chronic GVHD.
- Adequate bone marrow function indicated by ANC>1000/mm^3 and platelets>50,000/mm^3 without growth factors or transfusions
- Cardiac: No myocardial infarction within 6 months prior to enrollment or NYHA Class III or IV heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at screening must be documented by the investigator as not medically relevant.

3.1.9 The effects of IL-2 on the developing human fetus are unknown. For this reason and because chemotherapeutic agents are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.10 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Ongoing prednisone requirement >1 mg/kg/day (or equivalent).

3.2.2 Concurrent use of calcineurin-inhibitors plus sirolimus. Either agent alone is acceptable.

3.2.3 History of thrombotic microangiopathy, hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura.

3.2.4 Exposure to any new immunosuppressive medication in the 4 weeks prior to enrollment.

3.2.5 Extra-corporeal Photopheresis (ECP) or rituximab therapy within 4 weeks prior to enrollment

3.2.6 Any contraindication to ECP, i.e. contraindication to heparin or 8-MOP.

3.2.7 Post-transplant exposure to any novel immunosuppressive medication (e.g., alemtuzumab) within 100 days prior to enrollment.

3.2.8 Donor lymphocyte infusion within 100 days prior to enrollment.

3.2.9 Active malignant relapse.

3.2.10 Active uncontrolled infection.

3.2.11 Inability to comply with IL-2 treatment regimen.

3.2.12 Uncontrolled cardiac angina or symptomatic congestive heart failure (NYHA Class III or IV: Appendix C; section 17.3).

3.2.13 Organ transplant (allograft) recipient.

3.2.14 HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with the agents used after allogeneic HSCT. In addition, these individuals are at increased risk of lethal infections. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.15 Individuals with active hepatitis B or C are ineligible as they are at high risk of lethal treatment-related hepatotoxicity after HSCT.

3.2.16 Other investigational drugs within 4 weeks prior to enrollment, unless cleared by the Principal Investigator.

3.2.17 Pregnant women are excluded from this study because of the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother, breastfeeding should be discontinued.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

We do not restrict study access for any participant based on gender, ethnicity or socioeconomic status.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at [redacted] and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at [redacted]

Exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

4.3 General Guidelines for Other Participating Institutions

Please refer to Section 2.3 of the Data and Safety Monitoring Plan (Appendix I).

4.4 Registration Process for Other Participating Institutions

Please refer to Section 4.7 of the Data and Safety Monitoring Plan (Appendix I)

5. TREATMENT PLAN

Study therapy:

Each study participant will receive standard-of-care twice-weekly ECP for 16 weeks as per the transfusion medicine SOP. Briefly, ECP will be performed with the Therakos UVAR XTS or Cellex systems. Blood volume processed and 8-MOP dosing will be determined by subject hematocrit and blood volume per manufacturer guidelines. Clinical responders may continue ECP as standard-of-care after week 16, with or without extended-duration IL-2 therapy. If participants on ECP experience worsening of cGVHD requiring additional therapy prior to week 8, they may initiate low-dose IL-2 early, in consultation with the study PI.

After week 8, participants will initiate daily low-dose SC IL-2 (1×10^6 IU/m^2/day) for self-administration for the remaining 8 weeks of ECP, i.e. from end of week 8-16. IL-2 will be typically administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). Reference the extended-duration therapy section below for information concerning IL-2 therapy starting after Week 16.

Prednisone (or equivalent steroid) will be continued concomitantly with IL-2 without dose modification. Taper of prednisone will be permitted at the discretion of the treating physician if deemed in the participant's interest (e.g. steroid toxicity).

Of note, clinically stable cGVHD during taper of other immune suppression medications will be considered evidence of IL-2 efficacy; and progression of cGVHD during taper of other immunosuppressive therapy will not be considered evidence of IL-2 toxicity or lack of efficacy.

Reference Section 6.2 for instructions on dose modifications and delays of IL-2.

Extended-duration therapy:
After completing the 16 week study, patients experiencing clinical benefit (complete or partial response; as well as minor response not meeting NIH criteria for partial response) with an acceptable toxicity profile will be permitted to continue extended-duration IL-2 treatment indefinitely at the discretion of the treating physician. Restarting on extended-duration treatment can be delayed for only up to 2 weeks for justifiable clinical or administrative reasons. Longer delays in restarting treatment will have to be approved by the Principal Investigator. While on extended-duration IL-2, patients will be reassessed every 4 weeks to determine if IL-2 therapy should continue, at the discretion of the treating physician, who will document the rationale for the continued IL-2 therapy.

Toxicity data for extended-duration IL-2 will be collected on an ongoing basis and all treatment-related SAEs (per section 11.2) will be reported to the Principal Investigator and the IRB. Taper of other immune suppression medications during extended-duration IL-2 will be at the discretion of the treating physician. Addition of other cGVHD therapies to enhance response may be permitted for participants continuing on extended-duration therapy, at the discretion of the treating physician. While on extended-duration therapy, participants may have their dose modified in the event of toxicity per guidelines in Section 6.2 or at the discretion of the treating physician, in consultation with the Overall PI.
The required assessments for patients on extended-duration IL-2 therapy include:

1) Clinic visits locally or at study center for evaluation of toxicity and clinical benefit of IL-2 every 4 weeks (± 2 weeks). Required laboratory tests: CBC with differential; Serum Chemistries-glucose, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT, calcium.

2) Clinic visits at study center every 8 weeks (± 4 weeks). Required laboratory tests (in addition to above): immune assays that include quantitative serum immune globulins, plasma banking, and storage of additional mononuclear cells.

3) cGVHD assessments (Section 10.1) and cGVHD symptom score sheet every 16 weeks (± 4 weeks) until 1 year from the start of IL-2 treatment or the participant stops IL-2 therapy, whichever comes first.

5.1 Pre-treatment Criteria

5.1.1 The following evaluations must be performed within four weeks prior to ECP for all patients

- Medical history and documentation of the rationale for treatment of the patient's disease (including steroid dose).
- Physical examination, including vital signs, weight, performance status.
- cGVHD assessment (Section 10.1)
- Pregnancy test for women of childbearing potential.
- Hematology: Complete blood count (CBC) with differential.
- Serum chemistries: glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, AST, ALT, and calcium.
- Thyroid function tests (TSH, T4, free T4).
- CMV viral load
- Quantitative serum immune globulins
- Immunology: plasma banking; and storage of additional mononuclear cells.

5.1.2 The following evaluations are required (except where indicated) within four weeks prior to ECP for patients with cGVHD involving specific organ systems

- Ocular examination with a Schirmer's test, for patients with ocular cGVHD (optional).
- Dermatologic assessment (± biopsy), for patients with cutaneous cGVHD.
- Oral examination (± biopsy), for patients with oral cGVHD (optional).

- Pulmonary function tests, for patients with pulmonary manifestations of cGVHD (within 4 weeks prior to treatment to 1 week after).
- Flexion assessment of affected joints, for individuals with contractures or musculoskeletal involvement related to cGVHD.

5.1.3 Evaluations during ECP and IL-2 treatment (End of Weeks 1, 2, 4, 6, 8, 9, 10, 12, 14), and off-study (End of Week 16)

- Medical history and clinical examination (including steroid dose, weeks 8 & 16).
- Drug diary review with member of study team (starting with IL-2 treatment).
- Toxicity assessment will be done on the same day as history and clinical examination.
- Hematology: CBC with differential.
- Serum chemistries: glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, AST, ALT, and calcium.
- CMV Viral load (or per institutional practice).
- Quantitative serum immune globulins (weeks 4, 8, 12, 16).
- Immunology: plasma banking; and storage of additional mononuclear cells (weeks 1, 2, 4, 6, 8, 9, 10, 12, 14, 16)
- Thyroid function tests (TSH, T4, free-T4) (week 16)

5.1.4 The following assessments are required (except where indicated) at end of week 8 and 16 of study (in addition to cGVHD symptom score) for patients with cGVHD involving specific organs:

- cGVHD assessment (Section 10.1)
- Ocular examination with a Schirmer's test, for patients with ocular cGVHD (optional).

- Dermatologic assessment (± biopsy), for patients with cutaneous cGVHD.
- Oral examination (± biopsy), for patients with oral cGVHD (optional).
- Pulmonary function tests, for patients with pulmonary manifestations of cGVHD.
- Flexion assessment of affected joints, for individuals with contractures or musculoskeletal involvement related to cGVHD.

5.2 Agent Administration

5.2.1 IL-2

Recombinant human IL-2 (Aldesleukin) (Proleukin®-Novartis Inc. & Prometheus Labs, Inc.) for this study is supplied free of charge by Prometheus Laboratories, Inc.

5.2.2 Clinical Trial Materials

Recombinant human IL-2 (Proleukin®) is supplied as a sterile, white to off-white, lyophilized cake in single-use vials containing 22 MIU of aldesleukin intended for intravenous (IV) administration. Store vials of lyophilized IL-2 in a refrigerator at 2-8°C (36-46°F). Do not use beyond the expiration date printed on the label.

5.2.3 Preparation, Handling, and Storage of Study Drug

Please refer to section 7.1 for detailed information in this regard.

5.2.4 Drug administration and dosage schedule

IL-2 may be self-administered by daily subcutaneous injection. Teaching will be provided before the first subcutaneous injection (see Appendix J for instructions for subcutaneous injection).

The pharmacist, or the primary pharmacy technician working under the supervision of the pharmacist, will prepare the drug under aseptic conditions. The amount (in IU) of drug to be administered will be determined based on body surface area (BSA). BSA is to be calculated using the DuBois formula. (Appendix B; section 17.2). The dose should be calculated based on body weight at screening, at a dose of 1 x

10^6 IU/m^2/day. **Subsequent dose modifications, if any, will be per the guidelines in section 6.2.**

DO NOT ADMINISTER AS INTRAVENOUS PUSH OR BOLUS.

Premedications are not required prior to the first dose or later doses.

5.3 General Concomitant Medication and Supportive Care Guidelines

Antiviral, antifungal and antibacterial prophylaxis and monitoring should follow institutional practice for routine cGVHD management guidelines. These typically include: daily acyclovir (or equivalent) for HSV prophylaxis, bactrim (or equivalent) for PCP prophylaxis, IV gammaglobulin for hypo-gammaglobulinemia, and optional azole use for fungal prophylaxis in higher risk patients; as well as monitoring of CMV viral load, beta-glucan and galactomannan levels.

5.4 Duration of Therapy

Duration of therapy will be as per the schema outlined previously. In the absence of treatment delays due to adverse events, ECP plus IL-2 treatment may continue until one of the following criteria applies:

- The subject withdraws consent
- Non-compliance
- Administrative reasons
- Unacceptable adverse event
- Life threatening anaphylactic reaction to IL-2
- Other grade 4 toxic event (unless it is an asymptomatic correctable laboratory result, e.g. uric acid)
- Recurrent or non-resolving grade 3 toxic event
- Severe hematologic toxicity that persists or recurs (section 6.2).
- Life threatening infection on IL-2, at the discretion of the treating physician.
- Malignant relapse
- Clinical worsening of GVHD requiring the addition of a new immunosuppressive medication per the judgment of the treating physician. An increase in the corticosteroid dose will be considered evidence of worsening GVHD. Changes in other immunosuppressive medication doses to maintain a therapeutic level alone will not be criteria for removal from the trial.
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

5.5 Duration of Follow Up

Participants will be followed for 1 year from the start of ECP plus IL-2 therapy or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Participants will also be asked to allow for long-term follow up so that late toxicities, should they occur, may also be identified. Follow-up for participants benefiting on extended-duration therapy may continue beyond 1 year post start of IL-2.

Follow up will be at study center if patients live locally or with their local oncology providers if they live remotely. For patients living remotely, phone calls to their local oncology providers may be made on a 6 monthly basis.

5.6 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator.

John Koreth, MBBS DPhil,
[redacted]

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Version 4.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

ECP is a standard-of-care therapy and its adverse events, for instance, hemorrhage from heparin exposure, cytopenias, and procedure intolerance (i.e. constitutional symptoms or cardiovascular instability) are rare. [45] Apheresis physicians will evaluate for procedure-related toxicities per the transfusion medicine SOP. ECP may also exacerbate underlying constitutional symptoms or cardiovascular instability, e.g. in the setting of mild dehydration, or suboptimally controlled hypertension. Such exacerbations are rare and transient and routinely managed by apheresis physicians per the transfusion medicine SOP.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All CTCAE grade 3 and higher adverse events experienced by participants will be collected, through the study and until the final week 16 study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

The dose modifications in section 6.2 are recommended but not mandatory for participants proceeding on indefinite extended-duration IL-2 at the end of the 16 week study.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 Adverse Event List for IL-2

IL-2 (Proleukin) is a commercial agent. The relevant side effects of low-dose IL-2 in HSCT patients are described below. Additional detailed toxicity information may be found in the Proleukin package insert that relates primarily to high-dose IL-2.

- Local Reaction

Most patients receiving daily SC low-dose IL-2 reported injection site reactions, typically focal erythema that resolved in a few days; and induration that resolved after 2-3 weeks. Dose interruptions were occasionally required in patients with more marked induration (CTC grade 3). Lengthy dose interruptions may result in patients being unevaluable for response (Section 6.2)

- Constitutional symptoms
  In the HSCT setting, some patients on low-dose IL-2 developed fever, nausea and arthralgia within 72 hours of starting IL-2. Interruption of therapy resulted in symptom resolution with 3 days. Patients tolerated re-introduction of IL-2 at lower dose, with subsequent escalation to MTD dose.

- Thyroid dysfunction
  Thyroid function test abnormalities were noted in some HSCT patients on low-dose IL-2. Only one patient however developed clinical hypothyroidism necessitating therapy while on IL-2. After cessation of IL-2, thyroid function returned to normal. Hence, a thyroid panel (TSH, T4, free T4) levels will be checked at study entry and week 12 while on study. Patients with evidence for hypothyroidism will be worked up (antimicrosomal, antithyroglobulin antibodies) and given replacement thyroxine as clinically indicated.

- Hematopoiesis

  Early post-HSCT, low-dose IL-2 caused an initial decrease in the absolute lymphocyte count in most patients after 1 week of therapy. Thereafter, with continued infusion, a steady increase in lymphocyte count occurred in all patients. Low dose IL-2 also caused an initial increase in eosinophil counts (peak at 3 weeks) followed by a gradual decline. No changes in monocyte or neutrophil counts were observed.

  The platelet count decreased by >20% in some HSCT patients on low-dose IL-2. This decrease was noted within the first 2 weeks on IL-2, and continued treatment was not associated with further declines in platelet count. No patients had >40% decline in platelet counts, and none required platelet transfusions or had bleeding episodes.

  No significant impact of low-dose IL-2 on hemoglobin levels or reticuloctye counts was noted.

  Suggested Guidelines for the Management of severe hematologic toxicity are per section 6.2.

- Thrombotic Microangiopathy (TMA)

In DFCI 07-083, 2 patients on daily SC low-dose IL-2 developed SAE of thrombotic microangiopathy (thrombocytopenia, microangiopathic hemolytic anemia with schistocytosis, renal dysfunction) that was thought possibly related to IL-2. TMA is also a known complication of calcineurin-inhibitor and of sirolimus (both of which both the patients were on). One of the patients has required long-term hemodialysis for management of renal dysfunction.

6.1.2 Adverse Event Lists for Other Agents

There is toxicity related to cGVHD and to the immune suppressive medications used in its treatment. These toxicities are routinely managed by the study investigators. For a comprehensive list of adverse effects, please refer to the package inserts of the individual immune suppressive agents

6.2 Dose Modifications/Delays

Toxicities are to be assessed according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), Version 4.0 (Appendix H; section 17.8)

- Anaphylaxis: Life threatening anaphylaxis related to IL-2 requires discontinuation of IL-2.
- Thrombotic microangiopathy: TMA during IL-2 therapy requires discontinuation of IL-2 if it is associated with CNS dysfunction, renal dysfunction requiring hemodialysis/CVVH, or need for hospitalization.
- CTC Grade 4 toxicity: Grade 4 non-hematologic toxicity related to IL-2 requires discontinuation of IL-2, unless it is an asymptomatic correctable lab test abnormality (e.g. uric acid).
- CTC Grade 3 toxicity: IL-2 will be withheld for Grade 3 non-hematologic toxicities related to IL-2, unless it is an asymptomatic and correctable lab test result abnormality (e.g. uric acid). If the toxicity resolves to grade 1 or below within 2 weeks, IL-2 can be restarted at 50% dose that will not be re-escalated. If the toxicity does not resolve in 2 weeks to grade 1 or below, or recurs to grade 3 or above after restarting IL-2, it will be discontinued. IL-2 may also be withheld for Grade 3 non-hematologic toxicities unrelated to IL-2 per physician discretion. If withheld, subsequent management is suggested per above guidelines.
- Expected non-hematologic toxicity: IL-2 can be withheld and/or restarted at 50% dose for less than grade 3 toxicity (local site reactions, persistent constitutional symptoms) depending on patient tolerability and the discretion of the treating physician.

- Severe hematologic toxicity: IL-2 will be withheld for severe declines in peripheral counts (ANC<500, Plts<10,000) not related to malignant disease relapse, infection or other etiologies. If counts improve (ANC>1000, Plts>20,000) within 2 weeks, IL-2 will be restarted at 50% dose and not re-escalated. If peripheral counts do not improve within 2 weeks, or drop again (ANC<500, Plts<10,000) after restarting IL-2, it will be discontinued.
- Worsening of cGVHD: Clinical signs of worsening cGVHD while on IL-2 that require the addition of a new immunosuppressive medication (at the discretion of the treating physician), will be a criterion for IL-2 discontinuation. An increase in the corticosteroid dose above baseline will be considered evidence of worsening cGVHD. Changes in other immunosuppressive medication doses to maintain a therapeutic level alone will not be criteria for discontinuation of IL-2 or considered evidence of cGHVD worsening.
- Infection: Of note, infection during IL-2 treatment is not considered IL-2 related, since both cGVHD and the concurrent use of immune suppression medications are known risk factors for infection. Infection is therefore considered an expected complication of cGVHD. However, patients who develop a CTC grade 3 or higher infection prior to week 6 of IL-2 therapy will have IL-2 withheld. They can be considered for restarting IL-2 at their prior dose after control of infection, at the discretion of the treating physician.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 IL-2

7.1.1 Description

Native interleukin-2 (IL-2) is a secreted glycoprotein with a molecular weight of approximately 15 kilodalton (kD). The gene for IL-2 was isolated from the Jurkat leukemia cell line and expressed in *Escherichia Coli*. In contrast to native IL-2, recombinant IL-2 is non-glycosylated and differs at two amino acid positions. There are no discernable functional differences between native and recombinant forms of IL-2.

IL-2 pharmacokinetics has been evaluated in patients with cancer and with HIV. Following a short IV infusion, the pharmacokinetic profile of IL-2 was characterized by high plasma concentrations, rapid distribution into the extravascular space, and elimination from the body with a half-life of 1-2 hours. After SC administration, absorption of IL-2 was slow and the mean resident time was prolonged compared with IV administration. Maximum plasma concentration of IL-2 in HIV patients was achieved within 2-5 hours after SC dosing and the terminal phase half-life was estimated at 5 hours, suggesting prolonged absorption. Data on SC IL-2 administration suggest that approximately 35% is absorbed into the bloodstream, with greater bioavailablity (>60%) reported in HIV-infected participants.

There are at least 3 mechanisms of clearance of IL-2: glomerular filtration, peritubular extraction, and an inducible receptor-mediated mechanism (in man). Peri-tubular extraction of small peptides and proteins from the post-glomerular capillaries into the renal tubules and subsequent intracellular catabolism is a renal mechanism of elimination that occurs independent of glomerular filtration. Receptor-mediated clearance is primarily a function of IL-2 engaging its specific cellular receptor on responsive cells. The majority of IL-2 receptors are on T cells, and NK cells, though other cell types such as B cells have functional IL-2 receptors.

No pharmacologic interactions with other agents are anticipated in-vivo.

7.1.2 Form

Recombinant human IL-2 (Aldesleukin) (Proleukin®: Novartis Inc., & Prometheus Laboratories, Inc) is supplied as a sterile, white to off-white, lyophilized cake in single-use vials containing 22 MIU of aldesleukin.

For the 22 million international unit (MIU) vial, when reconstituted with 1.2 mL Sterile Water for Injection (SWFI), each mL contains 18 MIU (1.1mg) IL-2, 50 mg mannitol and ~180 mcg sodium dodecyl sulphate, buffered with ~170 mcg sodium phosphate monobasic and 890 mcg sodium phosphate dibasic to a pH of 7.5 (range: 7.2-7.8)

7.1.3 Storage and Stability

Store vials of lyophilized IL-2 in a refrigerator at 2-8°C (36-46°F). Do not use beyond the expiration date printed on the label. Vials should be entered only once for reconstitution to minimize the chances of contamination. If not used immediately, in-use storage times should normally not be longer than 24 hours at 2-8°C, unless reconstitution has been performed under controlled and validated aseptic conditions in a laminar airflow hood.

Data support stability and sterility of reconstituted diluted IL-2 preparations (reconstituted with SWFI and further diluted with D5W); and the stability and sterility of product reconstituted with SWFI but not further diluted, for up to 14 days at 2-8°C (36-46°F) when single-use syringes for daily use are prepared by qualified health-care professionals under aseptic conditions (per Proleukin® Investigator's Brochure (Chiron Corporation, 10 Sep 2003, pp113).

Therefore, reconstitution and dilution of lyophilized IL-2 is to be performed under controlled and validated aseptic conditions in a laminar flow hood per DFCI pharmacy policy and procedure manual . The dose or doses thus prepared and stored at 2-8°C (36-46°F) need to be used within 14 days.

7.1.4 Compatibility

IL-2 should be reconstituted with sterile water for injection (SWFI) plus D5W. Reconstitution or dilution with Bacteriostatic Water for Injection or 0.9% Sodium Chloride for Injection should be avoided due to increased aggregates.

IL-2 is not given IV, or in combination with other agents, hence compatibility issues are not applicable.

7.1.5 Handling

The single-daily-use syringes containing reconstituted and diluted solutions should be stored in a refrigerator at 2-8°C. DO NOT FREEZE. The product

should be inspected visually for particulate matter or discoloration and brought to room temperature before administration.

7.1.6 Availability

IL-2 (Proleukin®) is a commercial agent [redacted]

7.1.7 Preparation

IL-2 will be reconstituted in 1 ml Becton Dickinson single use syringes. All IL-2 [redacted]

[redacted]

7.1.8 Administration

After reconstitution, an up to 2-week IL-2 supply will be provided in single-use syringes (in a cool-pack if necessary), for home refrigerator storage at 2-8 C. One single-use syringe will be used each day during home SC self-administration, and discarded in the sharps' containers provided. Participants will be instructed to rotate injection sites, if feasible.

7.1.9 Ordering

[redacted]

[redacted]

7.1.10 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form.

7.1.11 Destruction and Return

At the end of the study, unused supplies of IL-2 should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8. CORRELATIVE/SPECIAL STUDIES

Investigational blood samples will be processed and banked with the Pasquarello Tissue Laboratory at DFCI. Please refer to the study operations manual for details regarding processing and handling of study specimens.

8.1 Pharmacokinetic Studies

N/A

8.2 Pharmacodynamic Studies

N/A

9. STUDY CALENDAR

Table 2: Summary of Required Data

	Within 4 Wks prior (baseline)	During Therapy (End of Wks 1, 2, 4, 6, 8, 9, 10, 12, 14)[a]	End of Wk 16[a]	Extended-duration IL-2 (4 Wkly)[c]
Medical History	X	X	X	X
Physical Exam	X	X	X	X
Toxicity Assessment		X	X	X
cGVHD Symptom Score	X	X[b]	X	X[#]
EKG	X			
Pregnancy Test[¶]	X			
Pulmonary Function	o	o[b]	o	o[#]
Dermatologic Assessment	o	o[b]	o	o[#]
Oral Assessment	o	o[b]	o	o[#]
Flexion Assessment	o	o[b]	o	o[#]
Ocular Assessment	o	o[b]	o	o[#]
CBC with Diff	X	X*	X	X
Serum Chemistry	X	X*	X	X
Quantitative Immune Globulins	X	X[d]	X	X[δ]
Immunology[1]	X	X	X	X[δ]
Steroid Assessment[2]	X	X[b]	X	X[δ]
CMV Viral Load	X	X	X	X
Thyroid Function	X		X	X[δ]
Drug Diary[+]		X	X	X

X- Required Evaluation

o- Required for patients with clinical involvement of these organ systems (oral and ocular assessments are optional), Baseline pulmonary function tests can be scheduled up to 4 weeks prior to start of treatment to 1 week after for scheduling flexibility.

1 Immunology: plasma banking; storage of additional mononuclear cells.

2 Systemic steroids should not be tapered unless there is toxicity, e.g severe hyperglycemia (with permission of study PI).

a Testing at end of weeks 1, 2, 4, 6, 8, 9, 10, 12, 14 will be performed ±4 days, to allow for scheduling and administrative flexibility around weekends, holidays etc.

b Week 8 for steroid dose and cGVHD assessments.

c Testing during extended-duration therapy will be performed ±2 weeks, to allow for scheduling and administrative flexibility around patient travel, work, holidays etc.

d Week 4, 8, 12.

* Laboratory testing of CBC/manual diff, serum creatinine and LDH will also be performed 4±2 days after IL-2 initiation, to assess for anemia, thrombocytopenia, schistocytes and/or renal dysfunction associated with thrombotic microangiopathy.

δ Every 8 weeks (±4 weeks) at time of mandatory study-center follow-up.

Every 16 weeks (+/- 4 weeks) during year 1 at time of mandatory study-center follow-up.

¶ For women of child-bearing potential.

+ To be completed and returned to clinic at least every 2 weeks during study IL-2 treatment. To be completed at and returned to clinic at least every 8 weeks for extended-duration IL-2.

10. MEASUREMENT OF EFFECT

Evaluable for toxicity- Participants who receive at least one dose of IL-2 will be evaluable for toxicity of IL-2 treatment.

Evaluable for response- Participants who have received at least 4 weeks of ECP+IL-2 and have had their disease re-assessed will be considered evaluable for combination ECP+IL-2 response. These participants will have their response classified according to the definitions stated below

10.1 cGVHD Assessments

Patients will undergo standardized cGVHD assessment per NIH guidelines (e.g., http://asbmt.affiniscape.com/associations/11741/files/ResponseCriteriaAPPENDIXAFormA.pdf) at baseline and week 8 and 16 on study. [46] cGVHD response will be assessed per NIH consensus criteria. [47] Of note, oral and ocular sites will not be included in determination of response, as additional topical therapy is permitted for those sites.

10.1.1 Complete response is defined as- Organ response: resolution of all reversible manifestations related to cGVHD in a specific organ; Overall response: resolution of all reversible manifestations in each organ or site of cGVHD involvement. Depending on relevant organ system involvement, patients will undergo repeat detailed assessment of ocular, oral, cutaneous, musculoskeletal and pulmonary systems.

10.1.2 Partial response is defined as- Organ response: at least 50% improvement in the scale used to measure disease manifestations related to cGVHD (e.g. a 50% decrease in skin rash from 80% BSA to 40% BSA), with a minimum of 25% improvement in the full scale as opposed solely to a percentage of the starting value (Appendix F; Section 17.6); Overall response: improvement in measure at least one organ or site, without progression in measures at any other organ or site. Of note, for global ratings and categorical scales, a 1-point change in a 3- or 7- point scale or a 2- to 3- change on a 0- to 10- point scale (0.5 SD change) would be considered clinically meaningful. Additionally, the hallmark for response to therapy for bronchiolitis obliteraans syndrome (BOS) is stabilization of lung function with no further decrease in FEV1 during a 3-month period.

10.1.3 Non-responders (e.g., minor response, stable disease) will not have changes in cGVHD meeting NIH criteria for partial response or disease progression.

10.1.4 Progressive disease- Organ progression: an absolute increase of at least 25% in the scale used to measure disease manifestations related to cGVHD (Appendix G; Section 17.7). Of note, for global ratings and categorical scales, a 1-point change in a 3- or 7- point scale or a 2- to 3- change on a 0- to 10- point scale (0.5 SD change) would be considered

clinically meaningful. Additionally, 'clinical worsening of cGVHD' is not synonymous with progressive cGVHD per NIH criteria, as patients may experience worsening symptoms that do not meet objective NIH criteria for progression. If so, they still have the option of discontinuation of IL-2 and initiating additional immunosuppression for lack of IL-2 efficacy; at the judgment of the treatment physician, as per section 6.2.

10.2 Chronic GVHD Symptom Score

Patients will self-report symptoms and signs of cGVHD using the validated chronic GVHD Symptom Scale (Appendix E; section 17.5). Self-Reported symptom Scales will be obtained at baseline and weeks 8 and 16.

10.3 Steroid Use for Chronic GVHD

Patients will have their total daily dose of corticosteroids recorded at baseline, and at 8 and 16 weeks. In the case of alternate daily dosing of corticosteroids, the average daily dose will be recorded for study purposes.

10.4 Immune Assessment

Patients will undergo testing for immunologic function, performed at baseline, and at weeks 1, 2, 4, 6, 8, 9, 10, 12, 14 and 16. Testing will include: plasma banking and storage of additional mononuclear cells. Quantitative immune globulins will be tested at baseline and weeks 4, 8, 12, and 16.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more sever form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or

convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of anticipated adverse events associated with IL-2.

11.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.

- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Procedures for AE and SAE Recording and Reporting

Reporting Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All CTC grade 3 or higher AEs and all SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the CTEP Version 4.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Version 4.0 of the CTCAE is identified and located on the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All appropriate treatment areas should have access to a copy of the CTEP Version 4.0 of CTCAE.

11.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

11.4.1 Serious Adverse Event Reporting

DF/HCC: All serious adverse events that occur after the initial dose of IL-2 study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) events that are unexpected and at least possibly related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) events that are unexpected and not specifically listed in the protocol as not requiring reporting. Grade 4 expected events related to stem cell transplantation that do not require reporting as SAEs include: neutropenia, neutropenic fever, thrombocytopenia, minor bleeding episodes (e.g. epistaxis), rashes, diarrhea, infections (e.g. pneumonia, line sepsis, cellulitis), VOD, TMA and GVHD as these are expected events related to stem cell transplantation.

- All Grade 5 (fatal) events while the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 business hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was

treated, and whether the participant will continue or discontinue study participation.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

The DF/HCC Principal Investigator will submit SAE reports from outside institutions to the DFCI Office for Human Research Studies (OHRS) according to DFCI IRB policies and procedures in reporting adverse events.

11.6 Reporting to Prometheus

A copy of any SAE report submitted to the DFCI IRB or FDA must be sent to Prometheus Laboratories. If the 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.

11.7 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to `institutional policy.

11.8 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the

study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet regularly to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 50 – Protection of Human Subjects www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html

- Title 21 Part 54 – Financial Disclosure by Clinical Investigators
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

- Title 21 Part 56 – Institutional Review Boards
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

- Title 21 Part 312 – Investigational New Drug Application
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws

- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Multi-center Guidelines

N/A

13.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

N/A.

14. STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

This is a one-stage Phase II trial to assess the efficacy of 16 weeks extracorporeal photopheresis (ECP) with the addition of daily low-dose subcutaneous (SC) interleukin-2 (IL-2) for weeks 8-16 in patients with steroid-refractory chronic graft-versus-host disease (cGVHD). The primary endpoint is overall response as defined in Section 10.0. Response will be evaluated at 16 weeks of treatment.

Our previous studies of low-dose IL-2 alone (DFCI#07-083, 11-149) for steroid refractory cGVHD showed a overall response rate of ~50-60%, and a randomized phase 2 trial of ECP vs. placebo showed a 40% response rate of skin cGVHD and 33% response rate of non-skin cGVHD)[43]. Conservatively extrapolating this information, we would consider ECP+IL-2 potentially efficacious if the overall response rate is 75% or higher and unworthy if 50% or lower. With 25 evaluable patients, if 17 or more patients respond, the treatment will be considered efficacious for the treatment of steroid refractory cGVHD.

With this design, the probability of concluding the treatment efficacious is 0.85 if the true but unknown response rate is 75% and 0.05 if the true rate is 50%. This decision rule is calculated using the exact binomial method. Table 1 below presents the operating characteristics of this design. Patients will be considered evaluable for response if they receive at least one week of ECP, which we anticipate to occur to all patients based on our prior experience. Patient will be replaced if the patient is removed from the study for reasons unrelated to the treatment (see Section 5.4).

Table 1. Operating Characteristics

	True but Unknown Response Rate					
	50%	55%	60%	65%	70%	75%
Prob(≥17 responses in 25)	0.05	0.13	0.27	0.47	0.68	0.85

14.2 Sample Size/Accrual Rate

14.2.1 **Sample Size**

The target accrual is 25 evaluable patients.

14.2.2 **Accrual**

Based on our prior studies of 07-083 and 11-149, we anticipate that the accrual will complete within two years. Patients will be followed for 12 months after completing study accrual.

Gender of subjects will not be used as a criterion for inclusion or exclusion in this study and there are no restrictions on the accrual of minorities. In 2013, 41% of all transplanted patients were women and approximately 10% of patients were minorities. Based on this self-reported ethnicity and gender in our transplant program in 2013, the anticipated accrual in subgroups defined by gender and race is summarized in the table below.

Accrual Targets

Ethnic Category	Sex/Gender				
	Females		Males		Total
Hispanic or Latino	1	+	1	=	2
Not Hispanic or Latino	9	+	14	=	23
Ethnic Category: Total of all subjects	10	+	15	=	25
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	1	+	1	=	2
Black or African American	1	+	1	=	2
Native Hawaiian or other Pacific Islander	0	+	0	=	
White	8	+	13	=	21
Racial Category: Total of all subjects	10	+	15	=	25

14.3 Stratification Factors

N/A

14.4 Analysis of Secondary Endpoints

Secondary endpoints include toxicity, immunologic effects, prednisone use, overall survival, progression-free survival, non-relapse mortality, and relapse. All analyses will be reported descriptively. Predictors for response will be explored in univariable analysis, including the impact of number of prior cGVHD therapies.

14.5 Monitoring for Adverse Events.

Adverse events will be monitored closely. The monitoring guidelines will serve as a trigger for consultation with the DF/HCC Data Safety and Monitoring Committee (DSMC) for additional review, and are not regarded as formal stopping rules that would mandate automatic closure of study enrollment. ECP+IL-2 treatment was well tolerated in the 4 DFCI patients treated thus far.

If in the first 10 patients who start the IL-2 treatment, we observe 3 or more patients with grade 3 or higher treatment related toxicity, further accrual will be halted pending review by the DSMC. Depending on the findings of its review, the DSMC may recommend the permanent closure of enrollment or continuation of enrollment. With this design, the probability of halting the enrollment is 0.07 if the true but unknown grade 3 or higher treatment related toxicity rate is 10%, 0.62 if the rate is 30%, and 0.95 if the rate is 50%.

The same rule will be applied to non-relapse mortality, relapse, and cGVHD progression.

15. PUBLICATION PLAN

The initial study results will be made public within 12 months of the end of data collection. The study report is planned for publication in a peer-reviewed journal, and the initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A final report of study outcomes will be made public no later than 3 years after the end of data collection.

The study PI holds the primary responsibility for publication of study results. Permission of the study PI will be required before any unpublished study information can be used or passed on to a third party.

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17. APPENDICES

17.1 Appendix A: PERFORMANCE STATUS

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

17.2 Appendix B: BODY SURFACE AREA AND CREATININE CLEARANCE

Body surface area (BSA) should be calculated using Dubois formula that yields the following results in meters squared (m^2):

$$BSA = (W^{0.425} \times H^{0.725}) \times 0.007184$$

where the weight is in kilograms and the height is in centimeters.

Creatinine clearance (CrCl) can be calculated using the Cockroft-Gault equation as follows:

$$\text{CrCl (ml/min)} = \frac{(140 - \text{age}) (\text{actual wt in kg})}{72 \times \text{serum creatinine (mg/dl)}}$$

For females, use 85% of calculated CrCl value.

Note: In markedly obese patients, the Cockroft-Gault formula will tend to overestimate the creatinine clearance. (Adipose tissue tends to contribute little creatinine requiring renal clearance.)

17.3 Appendix C: New York Heart Association Classification of Heart Disease

The following table presents the NYHA classification of cardiac disease.

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

17.4 Appendix D: DEFINITE AND PROBABLE cGVHD MANIFESTATIONS

Organ System	Definite manifestations of chronic GVHD	Possible manifestations of chronic GVHD
Skin	Scleroderma (superficial or fasciitis), lichen planus, vitiligo, scarring alopecia, hyperkeratosis pilaris, contractures from skin immobility, nail bed dysplasia	Eczematoid rash, dry skin, maculopapular rash, hair loss, hyperpigmentation
Mucous membranes	Lichen planus, non-infectious ulcers, corneal erosions/non-infectious conjunctivitis	Xerostomia, keratoconjunctivitis sicca
GI tract	Esophageal strictures, steatorrhea	Anorexia, malabsorption, weight loss, diarrhea, abdominal pain
Liver	None	Elevation of alkaline phosphatase, transaminitis, cholangitis, hyperbilirubinemia
GU tract	Vaginal stricture, lichen planus	Non-infectious vaginitis, vaginal atrophy
Musculoskeletal /Serosa	Non-septic arthritis, myositis, myasthenia, polyserositis, contractures from joint immobilization	Arthralgia
Hematologic	None	Thrombocytopenia, eosinophilia, autoimmune cytopenias
Lung	Bronchiolitis obliterans	Bronchiolitis obliterans with organizing pneumonia, interstitial pneumonitis

17.5 Appendix E: CHRONIC GVHD SYMPTOM SCORING SCALE

Name: MRN: Signature: Date:

Have you had any of the following problems in the past 2 weeks?

	Not at all	Slightly	Moderately	Quite a bit	Extremely
SKIN:					
a. Abnormal skin color	0	1	2	3	4
b. Rashes	0	1	2	3	4
c. Thickened skin	0	1	2	3	4
d. Sores on skin	0	1	2	3	4
e. Itchy skin	0	1	2	3	4
EYES AND MOUTH:					
f. Dry eyes	0	1	2	3	4
g. Need to use eye drops frequently	0	1	2	3	4
h. Difficulty seeing clearly	0	1	2	3	4
i. Need to avoid certain foods due to mouth pain	0	1	2	3	4
j. Ulcers in mouth	0	1	2	3	4
k. Receiving nutrition from an intravenous line or feeding tube	0	1	2	3	4
BREATHING:					
l. Frequent cough	0	1	2	3	4
m. Colored sputum	0	1	2	3	4
n. Shortness of breath with exercise	0	1	2	3	4
o. Shortness of breath at rest	0	1	2	3	4
p. Need to use oxygen	0	1	2	3	4
EATING AND DIGESTION:					
q. Difficulty swallowing solid foods	0	1	2	3	4
r. Difficulty swallowing liquids	0	1	2	3	4
s. Vomiting	0	1	2	3	4

t. Weight loss	0	1	2	3	4

	Not at all	Slightly	Moderately	Quite a bit	Extremely
MUSCLES AND JOINTS:					
u. Joint and muscle aches	0	1	2	3	4
v. Limited joint movement	0	1	2	3	4
w. Muscle cramps	0	1	2	3	4
x. Weak muscles	0	1	2	3	4
ENERGY:					
y. Loss of energy	0	1	2	3	4
z. Need to sleep more/take naps	0	1	2	3	4
Aa. Fevers	0	1	2	3	4
MENTAL AND EMOTIONAL:					
Bb. Depression	0	1	2	3	4
Cc. Anxiety	0	1	2	3	4
Dd. Difficulty sleeping	0	1	2	3	4

17.6 Appendix F: cGVHD PARTIAL RESPONSE CRITERIA

Suggested calculations for partial organ response in cGVHD

Organ and Starting Score or Value	Partial Response Criterion*
Skin (percent of body surface)	
> 50 %	e/s ≤ 0.5 and e > 0
25 – 50 %	s – e ≥ 25 and e > 0
< 25 %	only CR; no PR possible
Platelet count	e – s ≥ 100,000/uL and e < LLN
Gastrointestinal (and other 0 – 3 scales)	
3	e = 1 or 2
2	e = 1
1	only CR; no PR possible
Liver function tests (ALT, alkaline phosphatase and bilirubin)	
≥ 3x ULN	e/s ≤ 0.5 and e > ULN
< 3x ULN	only CR; no PR possible

*s, starting score or value; e, ending score or value; ULN, upper limit of normal; LLN, lower limit of normal

Examples
1. Skin: start score = 85, end score = 30; e/s = 30/85 = 0.35 = PR
2. Skin: start score = 65, end score = 45; e/s = 45/65 = 0.75 = not PR
3. Skin: start score = 45, end score = 15; s – e = 30 = PR
4. Skin: start score = 30, end score = 15; s – e = 15 = not PR

17.7 Appendix G: cGVHD PROGRESSION CRITERIA

Suggested calculations for organ progression in cGVHD

Organ and Starting Score or Value	Progression Criterion*
Skin (percent of body surface)	e – s ≥ 25
Platelet count	s – e ≥ 50,000/uL and e < LLN
Gastrointestinal (and other 0 – 3 scales)	e – s ≥ 1
Liver (ALT, alkaline phosphatase and bilirubin)	
s ≥ 3x ULN	e – s ≥ 3 x ULN
s < 3x ULN	e – s ≥ 2 x ULN
Lungs (12-point Lung Function Scale)¶	e – s ≥ 3†

*s, starting score or value; e, ending score or value; ULN, upper limit of normal

¶The lung function scale is the sum of the FEV1 and DLCO (corrected for Hb) scores, each computed as: > 80% of predicted = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6.

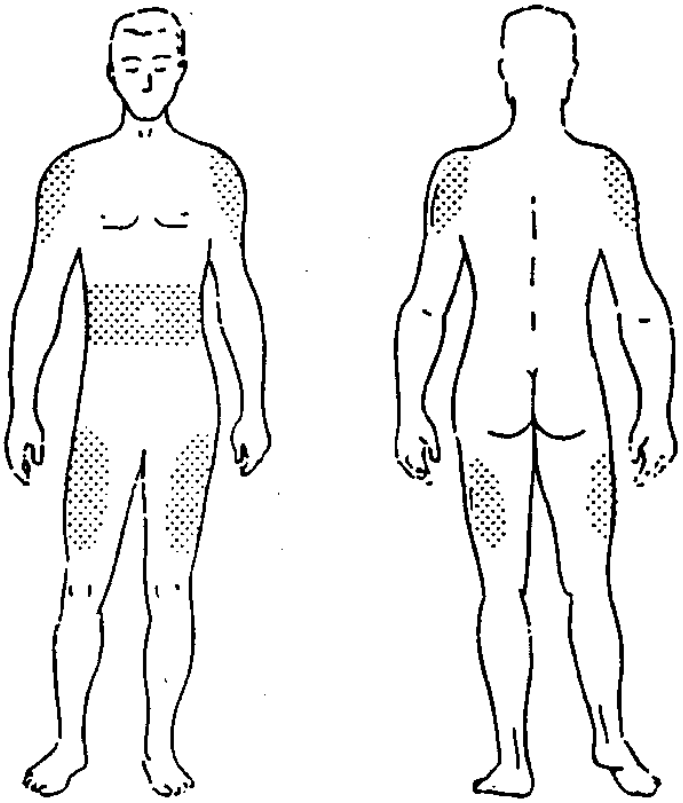
†If the starting lung function score is ≥ 10, progression is defined as ≥ 5% decrease of FEV1 in two tests measured at least 2 weeks apart. This time interval was selected because these syndromes can progress rapidly.

17.8 Appendix H: NCI COMMONTOXICITY CRITERIA

Please refer to the CTEP website: http://ctep.cancer.gov/reporting/ctc.html
Forms available upon request.

17.9 Appendix J: INSTRUCTIONS FOR SUBCUTANEOUS INJECTION/DRUG DIARY

INSTRUCTIONS FOR
SUBCUTANEOUS INJECTION



1. Once you have all your supplies, clean the work surface with isopropyl alcohol and wash your hands.

2. Remove the plastic cap or peel back the paper and attach the needle to the syringe without touching the hub of needle as you have been shown.

3. Wipe the top with an alcohol wipe.

4. Choose your injection site (see diagram). Rotate injection sites, if feasible. Avoid areas that are inflamed, edematous, scarred or covered by a mole, birthmark or other lesion.

5. Clean your skin with an alcohol wipe, using a circular motion, working outwards. Allow area to dry.

6. Remove the cap and hold the needle at a 45° angle (as you would a pencil). Gently grasp skin with one hand and quickly insert the needle with the other.

7. Pull back the plunger; if blood is seen in the syringe, remove the needle from the skin and change the needle to a clean one of the same size. It is alright to use the drug already in the syringe.

8. Inject the drug slowly.

9. Remove the needle and press the site gently with an alcohol swab or 2x2 gauze until the bleeding has stopped.

10. Dispose of the needle and syringe in the sharps' container provided.

If you have any questions, please call:

COMMENTS:

A Phase II Trial of Low-Dose Interleukin-2 (IL-2) Added to Extra-Corporeal Photopheresis for Refractory cGVHD

DOSING LOG

Study Participant
Self-Administration
Study Drug Diary
Dana-Farber/Harvard Cancer Center

Please indicate the date, if the contents of syringe were injected, and any comments.

	Date	Were contents of syringe injected?	Comments
Ex:	6/1/2009	Yes	
Day 1			
Day 2			
Day 3			
Day 4			
Day 5			
Day 6			
Day 7			
Day 8			
Day 9			
Day 10			
Day 11			
Day 12			
Day 13			
Day 14			

Study Participant Initials _____ Date _____

FOR STUDY TEAM USE ONLY	
Staff Initials:	
Date Dispensed:	
Discrepancy Notes:	

Participant Identifier: _____
Protocol # :
Your MD _____ Phone _____
Your RN _____ Phone _____

STUDY DRUG INSTRUCTIONS:

Study Drug: Interleukin-2
How Much: Your dose is 1.0 x 10^6 IU/m^2
How Often: You will self-administer each dose DAILY

SPECIAL INSTRUCTIONS:
An up to 2 week IL-2 supply will be provided in single-use syringes
IL-2 must be chilled for transit and for home refrigerator storage at 2-8 C.
One single-use syringe will be used each day during subcutaneous self-administration, and discarded in the sharps' containers provided.
Missed doses should not be made up.
Rotate your injection sites, if possible.
DO NOT FREEZE IL-2.