CITY OF HOPE NATIONAL MEDICAL CENTER
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DEPARTMENT OF HEMATOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: Phase II trial of Extracorporeal Photopheresis (ECP) plus Low Dose IL-2 for Treatment of Steroid Refractory Chronic Graft-Versus-Host Disease (cGVHD)

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 15125 **VERSION: 10**

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COH Amendment 08	Protocol Dated 07/31/2017	Version: 08
COH Amendment 09	Title Page Dated 07/20/2018	Version: 09
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SITE: Chronic GVHD involving skin, hepatic and GI Tract

STAGE: Steroid-refractory

MODALITY: Subcutaneous (IL-2) and apheresis (ECP

TYPE: Phase II

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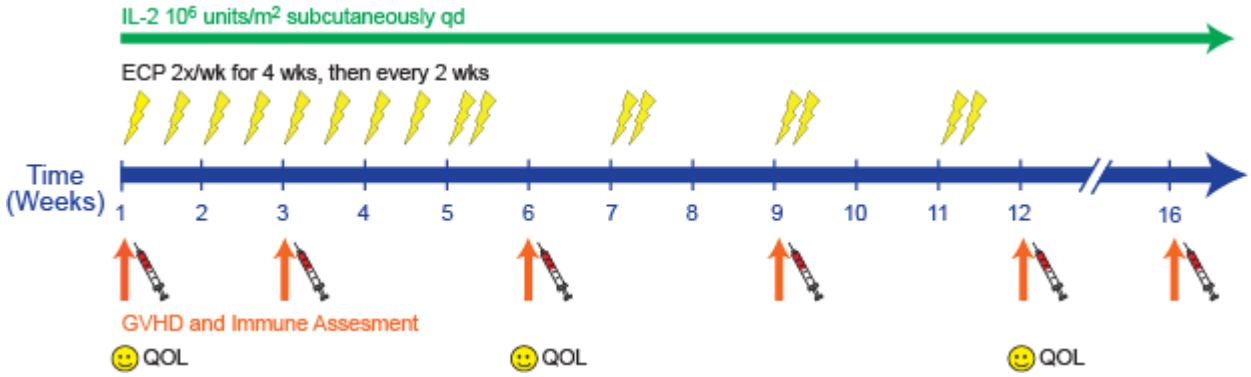
Experimental Design Schema

This single center/single arm, phase II clinical trial is designed to evaluate the anti-cGVHD activity of extracorporeal photopheresis (ECP) when combined with low dose IL-2, in patients with steroid refractory cGVHD, as assessed by overall cGVHD response rate (CR+PR+SD).



Protocol Synopsis

Protocol Title:
A Phase II trial of extracorporeal photopheresis (ECP) plus low dose IL-2 for the treatment of steroid refractory chronic graft-versus-host disease (cGVHD)
Brief Protocol Title for the Lay Public (if applicable):
Immunotherapy with IL-2 and ECP for refractory chronic graft versus host disease
Study Phase:
Phase II, non-randomized single institution trial.
Participating Sites:
City of Hope National Medical Center
Rationale for this Study:
Impaired immune reconstitution due to alteration in the ratio of regulatory T cells (Tregs) and conventional T cells (Tcon) post hematopoietic cell transplant is responsible for clinical manifestations of chronic GVHD. We propose that the combination of ECP and IL-2 will have a synergistic effect on increasing the proportion of Tregs, leading to normalization of Treg: Tcon ratio. This should lead to improved overall response rates (CR, PR and stable disease) when IL-2 and ECP are used in combination, compared to historical controls when either modality is used alone.
Objectives:
Primary: To evaluate the anti-cGVHD activity of extracorporeal photopheresis (ECP) when combined with low dose IL-2, in patients with steroid refractory cGVHD, as assessed by overall cGVHD response rate (CR+PR+SD). **Secondary:** • Characterize and evaluate toxicities, including type, frequency, severity, attribution, time course and duration. • Estimate overall and failure-free survival, non-relapse mortality (NRM) and relapse, through 1 year after initiation of treatment. • Characterize the chronic GVHD Symptom Scale scores • Determine ongoing prednisone use with IL-2 and ECP combination therapy. • Assess the immunologic effects of low-dose daily SC IL-2 + ECP. • Correlate clinical endpoints of response with ECP performance parameters.
Study Design:
Single-arm, single institution Phase II trial to estimate the activity of low dose IL-2 and ECP in patients with steroid refractory GVHD.
Endpoints:
Overall response rate to a 12-week course of subcutaneous low-dose daily IL-2 plus ECP in patients with steroid-refractory cGVHD. The primary endpoint of overall response is as defined in Section 11.0. Responses will be assessed at 3 weekly intervals while on IL-2 + ECP.
Sample Size:

The trial is expected to enroll 24 patients . Using a Simon two-stage design with type I error of 0.05 and 80% power a minimum of 24 patients are needed for enrollment to discriminate a promising response rate of 50% from current response rate of 25%

Estimated Duration of the Study

4years (per section 5.3)

Summary of Subject Eligibility Criteria:

Inclusion Criteria:

1. Recipients of allogeneic stem cell transplantation (sibling/unrelated/UCB/Haplo) with myeloablative or non-myeloablative conditioning regimens.
2. Participants must have steroid-refractory cGVHD. Steroid-refractory cGVHD is defined as having persistent signs and symptoms of cGVHD despite the use of prednisone at ≥ 0.20 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. 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.
4. Estimated life expectancy greater than 3 months.
5. Karnofsky performance status of 70-100 %.
6. Stable dose of corticosteroids for 2 weeks prior to enrollment.
7. Ability to understand and the willingness to sign a written informed consent document.
8. 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 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.
    - Adequate bone marrow function indicated by ANC>1000/mm3 and platelets>50,000/mm3 without growth factors or transfusions.

Exclusion Criteria:

1. Patients should not have any uncontrolled illness including ongoing or active infection. Ongoing prednisone requirement >1 mg/kg/day (or equivalent).
2. History of thrombotic microangiopathy, hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura.
3. Exposure to any new immunosuppressive medication in the 4 weeks prior to enrollment.
4. Donor lymphocyte infusion within 100 days prior to enrollment
5. Active malignant relapse.
6. Inability to comply with IL-2 treatment or ECP regimen.
7. Uncontrolled cardiac angina or symptomatic congestive heart failure (NYHA Class III or IV)
8. 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.

9. Patients may not be receiving any other parenteral investigational agents, or concurrent biological, chemotherapy, or radiation therapy. Oral chemotherapeutic agents or biologics –for example ruxolitinib therapy (either past or current exposure) - is allowed.
10. History of allergic reactions attributed to compounds of similar chemical or biologic composition to IL-2.
11. Patients must not have received prior chemotherapy (e.g. pentostatin) within 4weeks before study enrollment, or those who have not recovered from the adverse events due to agents administered more than 4 weeks earlier are excluded.
12. Pregnant women are excluded from this study. Because there is an unknown but potential riskfor adverse events in nursing infants secondary to treatment of the mother with IL-2, breastfeeding should be discontinued if the mother is treated with IL-2.
13. Patients with other active malignancies are ineligible for this study, other than superficial localized skin cancer (Basal or Squamous cell carcinoma).

Investigational Product Dosage and Administration:

Recombinant human IL-2 (Proleukin®) will be supplied as a sterile, lyophilized cake in single-use vials containing 22 MIU of aldesleukin intended for intravenous (IV) administration. The drug will be prepared by investigational pharmacy under aseptic conditions. The dose of 1 x 10^6 IU/m^2/day for 12 weeks will be calculated based on actual body weight at screening. IL-2 will be self-administered by daily subcutaneous injection and vials will be stored at 2-8 ºC. (36-46 degree F). ECP will be administered as per COH SOP. The drug will be provided by Prometheus labs and dispensed to patients by IDS pharmacy in prefilled syringes.

Clinical Observations and Tests to be Performed:

Patients will be evaluated for response to intervention (ECP + IL-2) at baseline and at weeks 3, 7, 9, 12 and 16. NIH clinical scoring forms will be used to document responses. Markers of immune activation will be monitored at the same time points from blood samples taken on scheduled clinic visits. Quality of Life Assessments will also be performed at baseline and in weeks 7 and 12.

Statistical Considerations:

The trial is expected to enroll 24 patients. Using a Simon two-stage design with type I error of 0.05 and 80% power a minimum of 24 patients are needed for enrollment to discriminate a promising response rate of 50% from current response rate of 25%

Sponsor/Licensee:

N/A

Case Report Forms

Medidata Rave EDC®

Table of Contents

Abbreviations

Abbreviation	Meaning
AE	Adverse Event
Allo-HCT	Allogeneic Hematopoietic Stem Cell Transplantation
COH	City of Hope
cGVHD	Chronic Graft-Versus-Host Disease
CR	Complete Response
CRC	Clinical Research Coordinator
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLT	Dose Limiting Toxicity
DSMC	Data Safety Monitoring Committee
ECP	Extracorporeal Photopheresis
FFS	Failure Free Survival
ICF	Informed Consent Form
IDS	Investigational Drug Services
IL-2	Interleukin-2
IND	Investigational New Drug
IRB	Institutional Review Board
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
PD	Progressive Disease
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
SAE	Serious Adverse Event
SC	Subcutaneous
SD	Stable Disease

1.0 Study Objectives

1.1 Primary Objective

To evaluate the anti-cGVHD activity of extracorporeal photopheresis (ECP) when combined with low dose IL-2, in patients with steroid refractory cGVHD, as assessed by overall cGVHD response rate (CR+PR+SD).

1.2 Secondary Objectives

- Characterize and evaluate toxicities, including type, frequency, severity, attribution, time course and duration.
- Estimate overall and failure-free survival, non-relapse mortality (NRM) and relapse, through 1 year after initiation of treatment.
- Characterize chronic GVHD Symptom Scale scores -self-report (with assistance from RNs and MDs).
- Determine ongoing prednisone use with IL-2 and ECP combination therapy.
- To assess efficacy of protocol therapy (IL-2+ECP) in helping to decrease the steroid dosage.
- Assess the immunologic effects of low-dose daily SC IL-2 + ECP.
- Correlate clinical endpoints of response with ECP performance parameters.

2.0 Background

2.1 Introduction/Rationale for Development

2.1.1 Chronic GVHD

Allogeneic hematopoietic stem cell transplantation (allo-HCT) is a proven effective immunotherapy for aggressive hematologic cancers that is used increasingly worldwide. Clinical outcomes post allo-HCT, including relapse, non-relapse mortality and overall long term survivorship have been improving for the past two decades and this is attributed to better donor selection, supportive care, less toxic conditioning regimens and advancements in management of acute and chronic graft versus host disease (GVHD)[1]. Despite these advances, allo-HCT continues to be associated with clinically significant morbidity and mortality, most of which is attributed to acute and chronic GVHD[2]. Glucocorticoids are the first line of therapy and responses are seen in about one-third of patients with cGVHD. Patients with cGVHD that is refractory to steroids are particularly difficult to treat as there is currently no standard of care. Effective therapies for steroid-refractory cGVHD are an unmet medical need and new strategies should be aimed towards alleviating symptoms and improving long term survivorship.

The underlying pathophysiologic mechanisms of cGVHD are complex and are related to the transplanted donor T-cells recognizing the recipient as "non-self" based on a variety of factors, the most important of which is degree of HLA disparity between donor-recipient pair. ChronicGVHD is comprised of an early initiating phase, followed by gradual evolution to the full blown syndrome, which can involve multiple organs. The "prime targets" are skin, GI tract and liver, which undergo tissue damage as part of the preceding chemotherapy and conditioning regimens. Other organs such as lung, salivary and lacrimal glands, oral mucosa, skin and subcutaneous tissues can also be involved and contribute to morbidity associated with cGVHD. During the initiation phase of cGVHD, recipient antigen presenting cells (rAPC's) engage Donor $CD8^+$ T cells by presenting self antigens in conjunction with the MHC class I complex. Donor $CD4^+$ T cells are activated by the MHC class II pathway, wherein, shed donor proteins and apoptotic cells are processed by endosomes and presented to Donor CD4+ cells by rAPC's. After antigen presentation and appropriate co-stimulatory signaling, donor $CD4^+$ and $CD8^+$ T cells undergo activation and expansion. In the evolution phase, donor APC's continue the ongoing activation of donor-

derived T cells by presenting recipient antigens via MHC class I and II peptides. The activated T cells cause tissue damage by release of cytokines (TNF-α, IL-2 IFN-γ) and by direct tissue infiltration[3].

Steroid refractory cGVHD is treated by a number of second-line immunosuppressive drugs including calcineurin inhibitors, sirolimus, mycophenolate mofetil, alemtuzumab, rituxan, tyrosine kinase inhibitors, proteosome inhibitors, etanercept and infliximab. There is no current standard of care for second-line therapy, so development of new agents and novel combinations of existing therapies for second-line GVHD therapy is an urgent need.

Evaluation of patients with cGVHD using validated and standardized response criteria is lacking at the present time and this is a major drawback while evaluating response to therapy across clinical trials. Inamoto et al.[4] recently described a new outcome measure, failure-free survival (FFS), to access response to second-line agents in patients with cGVHD. Failure events include non-relapse mortality, recurrent malignancy, and change in systemic therapy. FFS is a meaningful clinical metric that could be used as a shorter-term success end point for clinical trials. In their analysis of 312 patients with cGVHD who were on at least 0.5 mg/kg of prednisone equivalent and started on second-line immunosuppressive therapy, the 6 month FFS was 56%. The majority of failures were due to initiation of third line therapy (34%) indicating progression of cGVHD, 7 % were due to NRM and 3% due to disease relapse. Predictors of treatment failure were high-risk disease at HCT, lower GI involvement and high NIH global score at initiation of second-line therapy. FFS will be included as a secondary end point in our cohort.

2.1.2 Extracorporeal photopheresis (ECP)

Extracorporeal photopheresis (ECP) has emerged as a promising modality to treat steroid refractory cGVHD[5]. The procedure entails ex vivo treatment of leukapheresed patient lymphocytes with a photoactivatable compound and irradiation by ultraviolet A (UVA), followed by reinfusion of treated white blood cells (WBCs) back into the patient. ECP was initially applied in the treatment of cutaneous T-cell lymphoma (CTCL) and subsequently also in the treatment of acute and chronic graft-versus-host disease (GVHD), autoimmune disorders, and heart and lung transplant rejection. The clinical efficacy of ECP has been attributed to an immune modulatory effect and selective immune tolerance in some cases. The exact mechanism remains unknown; however, the following mechanisms of action are proposed;

- Clearance of apoptotic cells by antigen-presenting cells results in differentiation of those cells into a more tolerogenic phenotype leading to decreased stimulation of effector T cells or their deletion.
- Production of anti-inflammatory cytokines, especially interleukin 10, is increased
- Production of pro-inflammatory cytokines, especially interleukin 12 and TNFα, is decreased.
- Generation of CD4+, CD25+, GITR+, Foxp3+, CD62L+ T-regulatory cells.
- Generation of monocyte-derived, tolerogenic dendritic cells has been postulated to contribute to the downregulation of immune response in GVHD.

One study evaluated the percentage of functional T-regs after 6 ECP procedures demonstrating an increase from 8.9 to 29% of the total circulating CD4+ cells[6]. ECP is an apheresis-based therapy, where approximately 5 x 10^9 transplant-recipient leukocytes are treated with a photoactivatable 8-methoxypsoralen (8-MOP), followed by exposure to approximately 1.5 Joules/cm^2 of UVA light 0and reinfusion of treated leukocytes to recipients in a closed-loop system.

Flowers et al[7] reported a multicenter Phase II randomized study of ECP for patients with cGVHD. 95 patients with refractory cutaneous cGVHD were enrolled to this trial that compared standard therapy to standard therapy plus ECP given for 12-24 weeks. The median percentage improvement in total skin score (TSS) at week 12 was 14.5% for the ECP arm and 8.5% for the control arm (P= .48). No complete remissions were seen. The proportion of patients who had at least a 50% reduction in steroid dose and at least a 25% decrease from baseline in TSS was 8.3% in the ECP arm at week 12 and 0% in the control

arm (P = .04). The authors concluded that ECP was a well-tolerated modality and was associated with a significant steroid sparing effect. The short duration of therapy (12 weeks) contributed to the lack of statistically significant reductions in TSS and authors recommended longer duration of therapy for up to 24 weeks. Infections were the most serious side effect occurring in 18.4% of patients on ECP arm versus 26% in patients assigned standard therapy. Pneumonia and sepsis were the most common infectious complications. 2% mortality was noted in this study in the ECP arm and 6% in standard therapy arm. Improvements were also noted in extracutenaous sites of involvement including eye (30% ORR) and oral cGVHD (53%).

Malik et al.[5] reported a meta-analysis of 18 trials to access the efficacy of ECP in the steroid refractory cGVHD setting. Their analysis included one randomized controlled trial, two single arm prospective studies and the remainder were retrospective studies. The pooled complete response rates were 29% (CI, 19-42%) and overall response rates were 64% (CI, 65-82%). The responses varied according to organ involvement with response rates of 74%, 68%, 60%, 48%, 72% and 53% for skin, liver, ocular, lung, oral, and GI steroid refractory-cGVHD respectively. The main drawbacks in this study were the limited number of prospective trials and variability in duration and frequency of ECP schedules. Moreover there was variability in cGVHD assessment and endpoints. Despite these pitfalls, the authors concluded that the analysis supported the use of ECP in cGVHD especially in patients with cutaneous, oral and hepatic involvement.

Apisarnthanarax et al.[8] retrospectively analyzed their database of 32 Allogeneic HCT patients with steroid dependent (SD) and steroid resistant (SR) cutaneous cGVHD who were treated with ECP. Patients received a median of 36 ECP sessions over a median of 5.3 months. The complete response rate was 22% and partial response rate was 34%. 64% of patients who were steroid dependent were able to achieve a 50% reduction in steroid dosage while on ECP therapy. On long term follow up, 11patients (34%) have died due to progression of cGVHD symptoms or infectious complications. Durable CR's were seen in 5 of 7 patients. Patients who presented with progressive cGVHD fared worse that those presenting with de novo cGVHD.

In a recent meta-analysis by abu-Dalle[9] et al. the response rate of ECP after failure of corticosteroid therapy in chronic GVHD was reported as 64%, but responses varied by site of involvement with cGVHD; poor responses were noted in lung (15%). In this meta-analysis significant inter-trial differences were noted in organ-specific response rates and responses based on severity of GVHD were not reported.

In a prospective trial by Foss et al.[10], 25 patients with extensive steroid-refractory chronic GVHD with cutaneous and visceral involvement were assigned to once or twice weekly ECP therapy. The median duration of ECP therapy was 9 months. 20 patients with cutaneous cGVHD and 6 patients with oral cGVHD benefitted from therapy. Steroid sparing and reduction of systemic immunosuppressive therapy was possible in 80% of patients studied. Patients presenting with progressive cGVHD (with history of prior aGVHD) fared much worse that patients who presented with de novo cGVHD. Once or twice weekly ECP had similar outcomes. Overall response rate was reported as 71% for cutaneous cGVHD and 61% in visceral cGVHD. 13 patients developed serious adverse effects from ECP therapy including pneumonia (n=7),CMV colitis (n=1),GI bleed (n=1), urosepsis (n=1), line infection (n=1), catheter associated thrombus (n=1) and disease relapse (n=1).

There are few randomized controlled trials for use of ECP in cGVHD and most physicians use clinical experience and patient response to formulate institutional guidelines regarding frequency and duration of ECP usage. Based on in-house physician expertise in the COH Department of Transfusion Medicine, who have performed thousands of ECP procedures, we utilize an ECP schedule of: 2 procedures per week for the first month, followed by 2 procedures every 2 weeks for the next 3 months. For the purpose of this trial, we will continue using this regimen based on extensive local experience.

In conclusion, ECP is an important tool for use of therapy in steroid refractory and steroid dependent cGVHD with overall response rates of approximately 30% and partial responses in a majority of patients

treated. Responses depend on site of involvement with better outcomes in skin, hepatic, oral and ocular involvement. Patients presenting with de novo cGVHD, without history of preceding aGVHD also tend to respond better. Treatment responses improve with duration of therapy and most patients can gradually be tapered off ECP in 4-6 months. Treatment outcomes are better if instituted early in the course of cGVHD. However, enthusiasm for ECP in patients with cGVHD has to be tempered by the fact that large randomized controlled trials are lacking and few prospective trials have been done in this hard to treat patient population. Moreover, few patients achieve complete remissions, although a majority derives clinical benefit after ECP therapy from disease stabilization and partials responses. In most trials, ECP also helps patients to reduce the amount of steroid use. We believe that the complete and partial response rates should be improved upon by combining this therapy with other agents such as low dose IL-2.

2.1.3 Interleukin-2 (IL-2)

Interleukin-2 (IL-2) is a cytokine crucial to regulatory T cell (T-reg) proliferation, activation and survival[11]. It has been safely administered in patients without GVHD undergoing allo-HCT with T-cell depletion. IL-2 can augment the numbers of T-regs and NK cells without inducing GVHD.

T-reg cells ($CD4^+$, $CD25^+$, $FOXP3^+$) account for approximately 5 to 10% of circulating CD4+ T cells[12]. T-regs can modulate immune responses by suppressing auto- and allo-reactive lymphocytes, thus controlling innate and adaptive arms of the immune response. Loss of T-reg function is associated with autoimmune diseases and cGVHD[12]. Cell-mediated immunomodulation may be beneficial in patients with chronic GVHD. In preclinical models, adoptive transfer of T-reg cells has been shown to ameliorate GVHD, but the clinical application of this approach has been challenging. In patients with active cGVHD, low-dose IL-2 can enhance T-regs without increasing the relapse rate of the underlying malignancy and hence may have a role in tipping the balance of the immune system to a more tolerant state. Calcineurin inhibitors, which are standard therapy for most allo-HCT for prevention and treatment of chronic GVHD may impair T-reg expansion and delay immune reconstitution.

Koreth and colleagues[13] conducted a phase I trial of low-dose IL-2 for patients with steroid-refractory chronic GVHD. A total of 29 patients were enrolled. The highest dose level induced unacceptable constitutional symptoms. The maximum tolerated dose of interleukin-2 was 1×10^6 IU per square meter. Of the 23 patients who could be evaluated for response, 12 had major responses involving multiple sites. None had progression of chronic GVHD or relapse of a hematologic cancer. The numbers of CD4+ T-reg cells were preferentially increased in all patients, with a peak median value, at 4 weeks, that was more than eight times the baseline value ($P<0.001$), without affecting CD4+ conventional T cells (T-con). The T-reg:T-con ratio increased to a median of more than five times the baseline value ($P<0.001$). The T-reg cell count and T-reg:T-con ratio remained elevated at 8 weeks ($P<0.001$ for both comparisons with baseline values), then declined when the patients were no longer receiving interleukin-2. The increased numbers of T-reg cells expressed the transcription factor forkhead box P3 (FOXP3) and were able to inhibit autologous T-con cells. Immunologic and clinical responses were sustained in patients who received IL-2 for an extended period, permitting the glucocorticoid dose to be tapered by a mean of 60% (range, 25- 100). Additionally we have in house experience with use of single agent IL-2 (n=7) in patients who have been treated with ECP in the past. In these patients with steroid refractory cGVHD, all patients have derived clinical benefit, either partial response or stable disease, with no serious Grade III/IV adverse events noted at the dose level of 1 million Units /m^2

2.1.4 Rationale for combination of ECP and IL2 for cGVHD

As demonstrated in the literature reviewed above, cGVHD is a multisystem disorder caused by impaired immune reconstitution post Allo-HCT with allorecative T cells from donor causing immune mediated organ damage in skin, liver and GI tract. This condition is characterized by relative paucity of T regs

compared to Tcons leading to decline in Treg:Tcon ratio. Normalization of Treg:Tcon ratio is associated with objective clinical improvement in signs and symptoms of cGVHD. ECP has been used successfully in patients with chronic steroid-refractory GVHD, with complete responses noted in 30% of patients. Improvement in cGVHD symptoms in responding patients has been co-related with a concurrent increment in Tregs. Hence ECP is a proven treatment modality in patients with steroid refractory cGVHD and responding patients demonstrate a normalization of Treg;Tcon ratio.

More recently, IL-2 has shown promising results in a phase I dose escalation trial, wherein it was shown to be safe at a dose level of $1x10^6$ units/m^2 when used in patients with steroid refractory cGVHD. Clinical benefit (partial response and stable disease) 52% of patients on low dose IL-2 therapy. In this trial no complete responses were noted. Co-relative studies demonstrated that patients with cGVHD responding clinically had a significant expansion of T reg pool while on therapy, with responses noted as early as 2 weeks into therapy. This expansion of T regs persisted during the remaining duration of therapy and declined to baseline levels 4 weeks after discontinuation of subcutaneous IL-2. Relapse of cGVHD is noted after discontinuation of IL-2 therapy and this may be related to gradual decline in T regs after stopping IL-2 therapy.

In this phase II prospective trial in patients with steroid-refractory cGVHD, we propose to combine low dose IL-2 with ECP, based on the following:

A. We hypothesize that combination therapy with IL-2 and ECP will lead to a greater increase in Tregs compared to historic controls when either modality was alone. This is beaause both ECP and IL-2 boost regulatory T cells (Tregs). The effect of combining ECP and IL-2 on the Treg: Tcon ratio has not been studied in humans and correlative studies will be performed on all patients enrolled on this protocol to evaluate effect of combination therapy on normalizing immune reconstitution post-HCT in patients with steroid refractory cGVHD.
B. ECP and IL-2 have non-overlapping toxicities; hence, combination of the two should be well tolerated in patients with cGVHD. This combination is already being used off protocol(n=7) at COH after case review and approval by the GVHD committee in patients with cGVHD and the combination is well tolerated in patients treated so far. Patients on this combination have not experienced disease progression and all have derived clinic benefit from disease stabilization or partial responses.
C. Relapse of cGVHD is frequently noted after discontinuation of IL-2 therapy, possibly related to decline in Tregs to baseline after stopping low dose IL-2 therapy. After completion of protocol specified therapy at 12 weeks , responding patients will continue daily low dose subcutaneous IL-2 (commercial supply) and ECP (per SOP) till clinical benefit is no longer maintained. The decision to continue commercial supply of IL-2 will be per discretion of the treating primary physician in consultation with specialists in cGVHD clinic. No protocol specific monitoring or follow up data will be collected. Monitoring of these patients will be according to acceptable standard of care for patients on cytokine therapy. Ongoing responses will be monitored by the NIH consensus criteria guidelines, again off protocol. We hypothesize that maintenance therapy with IL-2 and ECP will maintain elevated T reg: Tcon ratio, thereby translating into fewer clinical relapses and improved clinical outcomes.

2.1.5 Immune correlative studies

Immune markers of cGVHD will be assessed in this trial using peripheral blood samples to correlate clinical response with changes in immune cell subsets. Immune cell subsets have been studied extensively in patients with cGVHD to determine their predictive value in chronic GVHD.

Most studies have focused on CD4+ (post-thymic) T cell subsets. Human peripheral blood CD4+ T cells are classified into 3 broad populations: i) naive CD45RA+ CCR7+, and 2 memory subsets, ii) CD45RA- CCR7+ (central memory), and iii) CD45RA- CCR7- (effector memory). Yamashita et al.[14] studied relative proportions of CD4 effector memory T cells (CCR7- $CD62L^{low)}$ in patients with established cGVHD and compared the distribution of this T cell subset in healthy controls and patients with no clinical signs of cGVHD. Chronic GVHD patients had a significantly higher percentage of CD4 effector memory T cells compared with healthy controls (35.5% vs 13.8 respectively; $P < .0001$) or stem cell transplantation patients without cGVHD (35.5 vs 21.7% respectively; $P < .01$) in the total CD4+T cell population. This finding indicates that effector memory T cells ($CD45RA^-$ $CCR7^-$ $CD62L^{low}$) may potentially be used as a marker of cGVHD.

Dander et al.[15] studied the role of IL-17-producing CD4+ T cells (TH_{17}) in cGVHD in the serum of 51 patients post allo-HCT with clinical manifestations of cGVHD, and compared this to 15 healthy donors (HD). Patients with cGVHD showed an increase of TH_{17} population compared with HD (mean SFU=178/25000 cells, n=18, ANOVA $P<0.001$). Importantly, by analyzing the proportion of TH_{17} cells according to the activation status of cGVHD (active vs. inactive phases), the authors were able to demonstrate that patients with active cGVHD, show an increase in the TH_{17} population (mean spot forming units [SFU] =237/25000 cells, n=13, ANOVA$P<0.001$). Inflammatory cytokines produced by TH_{17} cells - IL-6, TNF- α and IL-8 - were also significantly elevated in patients with active cGVHD. Hence the TH_{17} T cell subset by virtue of its secreted cytokines may be a useful adjunct to cGVHD assessment. In this analysis, CD 4+ T cells were selected by immunomagnetic beads using the Miltenyi system, plated in graded numbers and stimulated by 12-phorbol 13-myristic acid (50 ng/mL) and ionomycin (2g/mL) for 16 hr to elicit IFN-γ production.

T-regs constitute 5-10% of circulating $CD4^+$ T cells and suppress auto- and allo-reactive T cell clones. Loss of T-regs has also been associated with clinical symptoms of cGVHD. Phenotypically, T-regs are $CD4^+$ and $CD25^+$ and express forkhead transcription factor FOXP3. Zorn et al.[16] evaluated $CD4^+CD25^+$ T-regs in 30 patients with cGVHD after allogeneic HSCT, 27 patients without active cGVHD, and 26 healthy controls. They also evaluated TREC (T cell receptor excision circles) by peripheral blood PCR as a marker of thymic activity in post HCT patients. Patients with active cGVHD had significantly lower expression of FOXP3 when compared with patients without cGVHD ($P = 0.009$) or healthy donors ($P = 0.01$). Patients with cGVHD showed a significant decrease in TRECs compared with healthy donors ($P < 0.001$); supporting that thymic function is substantially impaired following allogeneic HCT.

Natural Killer cells (NK cells), B cells and T cell subsets were studied as part of immune reconstitution post allo-HCT prospectively by Abrahamsen et al.[17] in 57 patients. Blood and bone marrow samples were collected 3, 6, and 12 months after transplantation for analysis of immune reconstitution. Flow cytometric analysis was used for phenotyping T cell subsets. To assess the effect of chronic GVHD on immune reconstitution, patients with extensive cGVHD were compared to patients with no or limited chronic GVHD. The authors noted that extensive cGVHD is associated with elevated CD3+ T cells with relative decline in CD19+ B cells and $CD3^-CD16^+/56^+$ NK-cells.

Inflammatory biomarkers have also been used to follow patients with cGVHD. Dander et al.[15] studied peripheral blood samples obtained from 51 patients post allogeneic cell transplantation and patients developing GVHD were monitored for presence of TH-17 cells by ELISPOT or flow cytometry. The TH-17 cell population was increased (up to 4.8% of peripheral blood CD4+T lymphocytes) in patients with acute GVHD and (up to 2.4%) in patients with active chronic GVHD along with an inflammatory process. TNF-α was significantly increased in patients presenting with aGVHD (mean 49 pg/mL, range 3.6–176 pg/mL, n=10) and cGVHD (mean 28 pg/mL, range 4.8–77 pg/mL, n=14). Similarly, IL-6 levels were strongly enhanced in patients with aGVHD (mean 62 pg/mL, range 1–407 pg/mL) and active cGVHD. IL-8 cytokine levels were significantly elevated in patients with aGVHD (mean 20.8 pg/mL, range 3.9–49.1 pg/mL) and active cGVHD (mean 22.4 pg/mL, range 3.8–125 pg/mL) compared with healthy donors.

B cell activating factor (BAFF) is known to be a key regulator of normal B-cell homeostasis in humans[18]. Sarantopoulous et al.[19] studied 104 patients who had undergone allo-HCT between 1994 and 2005 for hematologic malignancies. ELISA was used to measure plasma BAFF levels and flow cytometry was used to assess BAFF receptor expression on B cells in patients with or without chronic GVHD. BAFF levels were significantly higher in patients with active chronic GVHD compared with those without disease ($P = 0.02$ and 0.0004, respectively). Patients treated with glucocorticoids showed reduction in BAFF levels indicating that this correlated with disease severity. Furthermore, it was noted that BAFF levels were high post HCT and declined in patients who never developed chronic GVHD. In contrast, BAFF levels remained elevated in patients who developed clinical manifestations of cGVHD. Six-month BAFF levels ≥10 ng/mL were strongly associated with subsequent development of chronic GVHD ($P < 0.0001$). Following transplant, plasma BAFF levels correlated inversely with BAFF receptor expression on B cells ($P = 0.01$), suggesting that soluble BAFF affected B cells through this receptor.

Rozmus et al.[20] prospectively studied mRNA levels of IFNγ, IL-2, IL-4 and IL-10 in peripheral blood mononuclear cells after non-specific mitogen stimulation with PMA/ionomycin or T cell stimulation with anti CD-3 in patients with cGVHD, and compared age matched controls of post-HCT patients without GVHD. In their analysis, early onset cGVHD was characterized by decreased expression of IFNγ and IL-2 mRNA after PMA/ionomycin stimulation. Late cGVHD was associated with decreased IL-2 and IL-4 mRNA expression after anti CD-3 antibody stimulation. Interestingly, elevated IFNγ mRNA expression predicted absence of early cGVHD and elevated IL-2 and IL-4 mRNA predicted absence of late cGVHD. Hence, early cGVHD was associated with decreased Th1 cytokine response and late cGVHD was associated with decreased Th2 cytokine response.

Recent studies also identified several biomarkers for acute and chronic GVHD as listed below.

1. IL-2 receptor α (IL2Ra), tumor necrosis factor receptor 1 (TNFR1), hepatocyte growth factor (HGF), interleukin 6 (IL-6) and interleukin-8 (IL-8) for systemic GVHD[21]
2. Elafin for skin GVHD[22]
3. Regenerating islet-derived 3α (REG3α) for gastrointestinal GVHD[21]
4. Suppression of tumorigenicity 2 (ST2) for steroid-refractoriness[23]
5. CXCL9 for chronic GVHD[24]

Based on this literature, inflammatory markers and immune cell subsets will be assessed in patients enrolled on protocol at baseline and at multiple time points during treatment with IL-2 + ECP as described in Section 9.0.

2.2 Overview of Proposed Study

In this Phase II prospective trial in patients with steroid refractory cGVHD, we propose to combine low dose IL-2 with ECP as both modalities promote immunologic tolerance by increasing the proportion of Tregs and the two therapies have non-overlapping toxicities. We anticipate that combination therapy will lead to better overall response rates (CR, PR and stable disease) compared to historical rates of either modality used alone. Eligible patients will receive 12 weeks of the combination therapy with daily IL-2 and ECP (administered per COH SOP), with clinical and immune evaluation every 3 weeks. Given the lack of effective therapies for steroid-refractory cGVHD, this prospective trial will help us evaluate the activity of this novel combination therapy in this difficult-to-treat patient population.

2.3 Preclinical Studies

Not applicable.

2.4 Human Studies

2.4.1 . Interleukin -2

2.4.1.1 IL-2 therapies in various diseases

Low-dose IL-2, administered subcutaneously (s.c.) for extended periods, has been evaluated in patients with HIV infection and cancer. In one study of ultra-low-dose IL-2[25], seven patients with HIV and non-Hodgkin lymphoma in first remission received 1 x 10^6 IU/m^2 daily of IL-2. After ~8 weeks of treatment, single-agent IL-2 therapy led to statistically significant, proportional increases in NK cells (1.6-fold) and T-regs (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.

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).

Low-dose IL-2 has also been safely used in patients with hematologic malignancy after allo-HCT. In one study, IL-2 was administered by continuous IV infusion to 29 asymptomatic patients after CD6+ T-cell depleted (TCD) allo-HCT at doses ranging from 2-6 x 10^5 IU/m^2/d, for periods of up to 3 months[26]. 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 T-regs. Further, a median ~8.5 fold increase of FOXP3 expression was noted, also indicating substantial T-reg enhancement.

2.4.1.2 Toxicity prolife of IL-2 in patients with cGVHD

The safety and immunologic activity of low-dose interleukin-2 in patients with active steroid-refractory cGVHD was assessed in a phase I trial, whose primary objective was to determine the MTD and toxicity profile of an 8-week course of IL-2[13]. Eligibility included patients with cGVHD requiring systemic therapy that had not responded to at least 0.25 mg/kg prednisone for a 4 week period, absence of infection, and stable doses of immune suppression for 4 weeks. The study had a Phase 1 dose escalation design with 3 dose levels (0.3, 1, 3; x 10^6 IU/m2/day for 56 days). 29 patients were accrued on protocol with subjects, 28 were evaluable for toxicity and 23 were evaluable for response. The interval between onset of chronic GVHD and initiation of IL-2 was 803 days (range, 117-2624). Patients were on a median of 3 concurrent agents (range, 1-3) for cGVHD. Skin (26 patients) and fascia (16 patients) were primary sites of involvement, but lung, liver, oral, and ocular sites were involved as well. Dose-level-C (3 x 10^6 IU/m^2/day) was declared unsafe due to constitutional side-effects (fever, malaise, and arthralgia). Dose-level-B (1 x 10^6 IU/m^2/day) was determined to be maximum-tolerated-dose in this patient population. Two patients developed dose-limiting-toxicity (thrombotic microangiopathy). CTCAE Grade IV toxicities included: TTP/HUS n=2; Dyspnea n=1, myocardial infarction n=1 and MRSA Pneumonia n=1. Grade III toxicities included induration n=3, lower GI Bleed n=1, deep vein thrombosis n=1, MRSA abscess n=1, and hemophilus bacteremia =1. Grade II toxicities included: constitutional symptoms n=1, increase in serum creatinine n=1, thrombocytopenia n=1. None experienced GVHD flare. There was no malignant disease relapse. 12/23 patients had major responses. These results suggest that low-dose IL-2 given at 1 x 10^6 IU/m^2/day for 8 weeks can be administered safely in patients with active chronic GVHD.

2.4.2 Extracorporeal Photopheresis:

2.4.2.1 *Initial use of ECP:*

Phototherapy involves the use of psoralen followed by exposure of skin to UVA radiation (PUVA). This form of treatment has been used for many years to treat cutaneous conditions such as vitiligo, eczema and psoriasis. ECP is a therapeutic procedure in which peripheral blood mononuclear cells are extracted by apheresis and exposed to 8-methoxypsoralen (8-MOP) followed by ultraviolet A (UVA) radiation. It was initially developed as an immunomodulatory therapy for treatment of cutaneous T cell Lymphoma (CTCL). This treatment damages DNA and induces apoptosis in circulating lymphocytes[27]. Edelson et al.[28] first reported on the use of this therapy in 37 patients with CTCL treated with oral methoxsalen followed by ex vivo photoactivation of lymphocytes with ultraviolet A (UVA) light. 27 of the original 37 patients responded to therapy with 64 % decline in cutaneous involvement after a mean of 22 weeks of therapy. Currently, ECP is used worldwide in the treatment of a variety of clinical conditions such as acute allograft rejection after cardiac, lung, renal and liver transplantation[29]. It is also used for treating steroid refractory acute and chronic graft versus host disease. ECP has also been used to treat a variety of autoimmune conditions including, scleroderma, rheumatoid arthritis, SLE and pemphigus vulgaris[30]. The role of ECP has been established in patients with steroid refractory and steroid dependent cGVHD. Please refer to the discussion presented in section 2.1.2 for further details.

The equipment for photopheresis was developed by Therakos, a Johnson and Johnson company (Raritan, NJ, U.S.A.), based on Latham bowl technology. Much of the reported use of photopheresis has involved a second-generation model—namely, the Therakos Uvar XTS system. This discontinuous, but completely contained, automated procedure collects some 5%–8% of circulating white blood cells into a plastic bag. A UV-A photosensitizing agent, 8-methoxypsoralen, is added to the bag, and the treated cells are then subjected to 1.5 J of UVA light. Afterwards, the cells are re-infused into the patient. The difficulty with this technology is its discontinuity.

To collect the leukocytes, the bowl first has to fill with red blood cells. There are two bowl sizes: 125 mL and 225 mL. If the patient's hematocrit is low, the volume of blood needed to fill the bowl with red blood cells may be very large. It is recommended that the extracorporeal volume not exceed the total blood volume by more than 15%. A third-generation apparatus has now been introduced by Therakos. The Therakos Cellex still uses the Latham bowl technology, but its single- or double-needle continuous-flow system is completely automated. The tubing volume for the single-needle procedure is 260 mL; for the double-needle procedure, it is 216 mL. The average treatment time for a patient was 180 minutes using the Therakos Uvar XTS. With the new apparatus, a single-needle procedure takes 100 minutes, and a dual-needle system, only 75 minutes[31]. At City of Hope both Cellex and XTS devices are available.

2.4.2.2 Toxicity prolife of ECP in patients with cGVHD

Toxicity rates vary in studies describing ECP therapy for chronic GVHD. In their review of ECP use in patients with cGVHD undergoing ECP, Abu-Dalle et al.[9] reported an incidence of 0.38% (95% CI, 0.06-0.78) for any grade 3 or 4 adverse events. Seaton et al.[32] reported severe complications in 5 of 28 subjects who received ECP for treatment of chronic GVHD: renal failure/sepsis, n = 1; acute respiratory distress syndrome of unknown cause, n = 1; pneumothorax/pleural effusion, n = 1; ischemic heart disease, n = 1; and unspecified, n = 1. Foss et al.[10] described serious adverse events in 15 of 25 subjects treated with ECP, specifically pneumonia, n = 5; cytomegalovirus colitis, n = 1; gastrointestinal bleeding, n = 1; urosepsis, n = 1; line-related complications, n = 2 (line sepsis, n = 1; deep venous thrombosis, n = 1); and unspecified, n = 5. In a randomized trial reported by Flowers et al[7], serious adverse events were reported in 28.6% (n=14) patients on the ECP arm and 26% (n=13) in patients randomized to the control arm. (*P-0.78).* In patients randomized to the ECP arm , infections were the most common side effects noted in 18.4% patients. Pneumonia (4.1%) and sepsis (4.1%) were the leading infectious complications. The majority of patients on the ECP arm (90%) developed adverse events (Grade 1 or 2) compared to 92% on control arm (*P-0.78).* These included diarrhea (20.4%), anemia (24.5%) and nausea (18.4%). Adverse

events that led to withdrawal from the ECP arm included thrombocytopenia, hypoglycemic coma, tremor, mental status changes, progressive GVHD, *Pseudomonas* sp lung infection, and catheter-related complications (1 patient each). In the control arm, patients discontinued participation in the study because of progressive tendinous contracture, bacterial (*Pseudomonas* sp) pneumonia, or fungal pneumonia (1 patient each).

In conclusion, ECP use is associated with serious adverse events in approximately 30 % of patients treated; however this is not significantly more that complication rates noted in control patients on standard GVHD therapies. The reason for high rates of infectious complications noted in this group of patients is that they are immunocompromised given prolonged exposure to steroids and other immunosuppressive agents used to treat cGVHD. Pneumonia and sepsis are the most important infectious complications –the latter complication seen most likely due to frequent central line access for ECP.

Most of the studies that have been reported on use of ECP in cGVHD were published from 1993-2010. Supportive care has improved upon since then - with better understanding of infection risk, improved infection surveillance and newer antimicrobials. These changes have led to improvements in non-relapse mortality and hence improved survival of patients [1]

Patients enrolled on this trial will be on standard prophylaxis with bactrim (or mepron for sulfa allergic patients) and acyclovir for prevention of PJP (Pneumocystis jiroveci pneumonia) and herpes zoster reactivation respectively. Patients who become neutropenic (ANC < 1000) will receive growth factor support with G-CSF and antibiotic (levofloxacin) prophylaxis. Central line care will be according to SOP mentioned under guidelines for supportive care of the HCT patient (D.001.05). See supportive care section 5.6 for further details.

3.0 Patient Eligibility

3.1 Inclusion Criteria

3.1.1 Disease Status

- Recipients of allogeneic stem cell transplantation with myeloablative or non-myeloablative conditioning regimens. Alternative donor transplants (Umbilical cord blood and haploidentical) are allowed.
- Patients with chronic GVHD requiring systemic therapy are eligible.
- Participants must have steroid-refractory cGVHD, which is defined as having persistent signs and symptoms of cGVHD despite the use of prednisone at 0.20 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.

3.1.2 Age Criteria, Performance Status and Life Expectancy

- 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.
- Karnofsky performance status of 70-100 %.
- Estimated life expectancy greater than 3 months.

3.1.3 Child Bearing Potential

The effects of IL-2 on the developing fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for six months following duration of study participation. Should a

woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

3.1.4 Protocol-Specific Criteria

- Stable dose of corticosteroids for 2 weeks prior to enrollment, i.e. the patient's steroid dose (mg/kg) will remain unchanged (eg 0.5 mg/kg) in the 2 weeks preceding enrollment. Allowances will be made for up or down titrating the dose based on changes in body weight.
- 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. 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.
  - *Adequate bone marrow function*: indicated by ANC>1000/mm^3 and platelets>50,000/mm^3.

3.1.5 Informed Consent/Assent

All subjects must have the ability to understand and the willingness to sign a written informed consent.

3.1.6 Prior Therapy:

Patients with steroid refractory cGVHD typically have received salvage with multiple lines of therapy; hence in this trial there will be no restriction in terms of prior lines of therapy received. Prior ECP exposure is allowed, however prior IL-2 use is excluded.

Exclusion Criteria

- Patients should not have any uncontrolled illness including ongoing or active infection. Patients with an ongoing prednisone requirement of >1 mg/kg/day (or equivalent) will be excluded.
- History of thrombotic microangiopathy, hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura.
- Exposure to any new immunosuppressive medication in the 4 weeks prior to enrollment.
- Donor lymphocyte infusion within 100 days prior to enrollment
- Active malignant relapse.
- Uncontrolled cardiac angina or symptomatic congestive heart failure (NYHA Class III or IV)
- HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with the agents used after allogeneic HCT. In addition, these individuals are at increased risk of lethal infections.
- Patients may not be receiving any other investigational agents, or concurrent parenteral biological, chemotherapy, or radiation therapy. Oral chemotherapeutic agents or biologics – for example ruxolitinib therapy (either past or current exposure) - is allowed.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to IL-2.
- Patients must not have received prior chemotherapy (pentostatin) within 4weeks before study enrollment, and those who have not recovered from the adverse events due to agents administered more than 4 weeks earlier are excluded.

- Pregnant women are excluded from this study because IL-2 is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with IL-2, breastfeeding should be discontinued if the mother is treated with IL-2.
- Patients with other active malignancies are ineligible for this study, other than superficial localized skin cancer (basal or squamous cell carcinoma).

3.2 Non-Compliance

Subjects, who in the opinion of the investigator may not be able to comply with IL-2 or ECP treatment requirements or the safety monitoring requirements of the study, will be excluded from participation .

3.3 Inclusion of Women and Minorities

The study is open to anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial that will accrue between 17-37 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

4.0 Screening and Registration Procedures

4.1 Screening Procedures

The following evaluations must be performed within four weeks prior to IL-2 +ECP treatment for all patients on protocol:

- Medical history and documentation of rationale for enrollment on IL-2 + ECP therapy on protocol.
- Physical examination, documentation of vital signs and KPS.
- Chronic GVHD assessment based on NIH consensus criteria – see Appendix C.
- Baseline lab work including CBC with differential, CMP and TSH/Vitamin D screen.
- Serum samples and mononuclear cells to be collected for immune assessment.
- Pregnancy test for women of childbearing age.
- Pulmonary function tests for patients with pulmonary manifestations of cGVHD.
- Dermatologic assessment for patients with cutaneous cGVHD.
- Flexion assessment of affected joints for individuals with contractures or musculoskeletal involvement secondary to cGVHD.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record.

4.3 Registration Requirements/Process

Following informed consent, the patient will be registered through the Clinical Trials Office (CTO) 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.

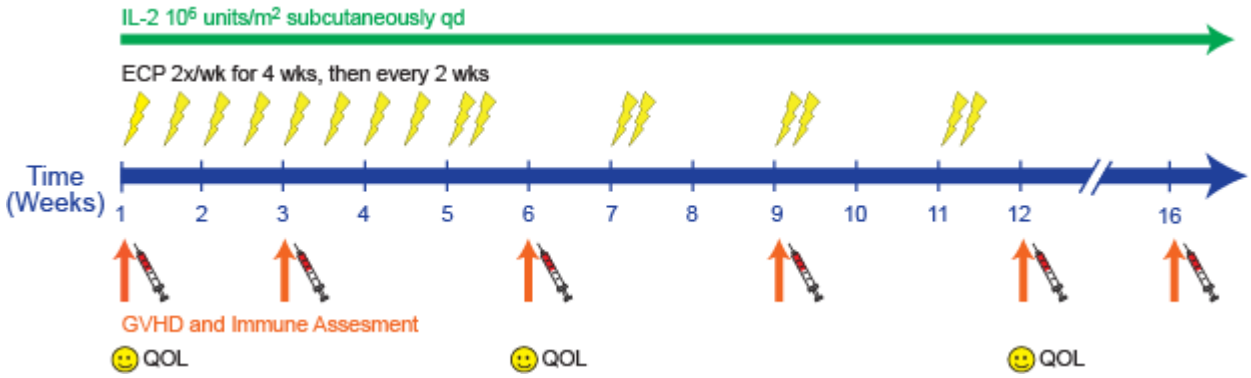
Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, notify the CTO of participant status changes as soon as possible.

4.4 Randomization and/or Dose Level Assignment

NA. This is a phase II single-arm trial. There is no dose escalation or randomization.

5.0 Treatment Program

5.1 Treatment Overview



GVHD and Immune Assessment and QOL will be performed at week 7 instead of week 6. (NB: Patients deriving clinical benefit after initial 12 week therapy have the option to go on a maintenance phase .

5.2 Treatment Schedule

5.2.1 IL-2 Therapy

D. IL-2 will be self-administered by patients as a daily subcutaneous injection. Teaching will be provided before the first 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). The dose should be calculated based on body weight at screening, at a dose of 1 x 10^6 IU/m^2/day for 12 weeks. All treatments will be outpatient based. If however, patients live within a 15 mile distance of the medical center and /or insurance mandates that the IL-2 be given in physician office- IL-2 injections will be given in the infusion center. After completion of 12 weeks of protocol therapy, responding patients will have the option of continuation of therapy till clinical benefit is no longer maintained. IL-2 will be provided by Prometheus Labs for this study. No protocol specific monitoring or follow up data will be collected after initial 12 week period. Monitoring of these patients will be according to acceptable standards of care for patients on cytokine therapy with IL-2. Ongoing responses will be monitored by the NIH consensus criteria guidelines , again off protocol . We hypothesize that maintenance therapy with IL-2 and ECP will

maintain elevated T reg: Tcon ratio, thereby translating into fewer clinical relapses and improved clinical outcomes.

5.2.2 ECP Therapy

ECP will be performed on an outpatient basis in the Amini Building at COH medical Campus according to the following schedule. All patients will have baseline evaluation in the cGVHD clinic prior to initiation of ECP.

- Weeks 1-4: twice weekly ECP
- Weeks 5-12: two ECP treatments every two weeks (weeks 5,7,9 and 11)

ECP treatments will be preferably given on consecutive days of the week (eg Monday and Tuesday) however this is not always possible. Schedule will be determined based on availability of ECP in the Apheresis center.

5.2.3 Observations and Tests to be Performed

Patients will be evaluated for response to intervention (ECP + IL-2) at baseline and at weeks 37, 9, 12 and 16. NIH clinical scoring forms will be used to document responses. Markers of immune activation will be monitored at the same time points from blood samples taken on scheduled clinic visits. Quality of Life Assessments will also be performed at baseline and in weeks 7 and 12.

5.2.4 Documentation of compliance to therapy

Each participant will be given explicit instructions for taking IL-2 subcutaneous injections at home. .Compliance to IL-2 home therapy will be closely monitored while they are on protocol therapy and patient will be given detailed instructions by the physician and research nurse in terms of administration of IL-2 subcutaneously and anticipated side effects . All enrolled patients will be given a drug diary (provided by the research nurse) at enrollment where they will document daily drug use and any missed doses and reasons thereof. The diary will be returned to the Research Nurse every 2 weeks while on protocol therapy and a new one will be issued at the same time.. IL-2 typically is supplied in 22 X 10^6 units multidose vials and these will be individually dispensed in single dose syringes to enrolled patients every 2 weeks for home administration.

DO NOT ADMINISTER IL-2 AS INTRAVENOUS PUSH OR BOLUS.

Pre-medications are not required prior to the first dose or later doses.

All treatment including IL-2 administration and ECP procedures will be conducted in the outpatient setting, unless patient is hospitalized for some other medical condition unrelated to underlying hematologic disease or adverse effects of therapy (e.g. therapy will continue while inpatient for pain control, failure to thrive etc.). ICU admission, respiratory and hemodynamic compromise will be grounds for withholding therapy. See section 6.0 for dose modification and stoppage rules.

Prednisone (or equivalent steroid) will be continued concomitantly with IL-2 without dose modification. Taper of prednisone, or any other immune suppression medications, will not be permitted during the initial 6 weeks of the study, but can be reduced thereafter in responders at the discretion of the treating physician.

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.

5.3 Planned Duration of Therapy:

IL-2 and ECP combination therapy will be given for 12 weeks, with final immune assessment occurring at 16 weeks post-initiation of therapy. Patients deemed to be benefiting from combination therapy (i.e. in complete remission, partial remission, minor response or stable disease), will have the options to continue onto the extended duration per discretion of treating physician.

5.4 Optional Extended-duration therapy:

As mentioned in the section above, patients who are responding to upfront therapy with IL-2 and ECP will be eligible to continue combination therapy with daily subQ IL-2 and ECP per SOP guidelines till clinical benefit is no longer maintained or toxicities develop. While on the optional maintenance therapy,these patients will be monitored for response and toxicity profile in the GVHD clinic on a monthly basis. This monitoring will not require data collection from research staff for response or adverse effect monitoring. Best clinical judgment will be used to determine on each monthly visit on rationale to continue commercial supply IL-2 or not based on clinical benefit and adverse effect profile of IL-2.

5.5 Criteria for Removal from Treatment

- Subject withdraws consent
- Non-compliance
- Administrative reasons (insurance loss or no coverage of IL-2 or ECP by third party payors)
- Any Grade 4 non hematologic adverse event
- Life threatening anaphylactic reaction to IL-2
- Recurrent or non-resolving grade 3 toxic event
- Malignant relapse
- Clinical worsening of GVHD requiring the addition of a new immunosuppressive medication. A 50% 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.

5.6 Subject Follow-Up

While on the first twelve weeks of therapy, participants will be undergo clinical (including cGVHD) and immunologic evaluations every 3 weeks. cGVHD severity/response will be assessed using the NIH scoring criteria (see Appendix).

Toxicity assessments will be performed using the CTEP Version 4.03 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.

If possible, adverse effects 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 from the time of the first dose of study treatment, through the study and until the final study visit.

Participants removed from the study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Follow-up for participants benefiting on extended-duration therapy will continue on monthly basis in the GVHD clinic.

5.7 Supportive Care, Other Concomitant Therapy, Prohibited Medications

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.

5.8 Additional Studies:

Baseline evaluations, tests or exams are valid 3 weeks prior to the start of treatment.

At baseline, weeks 3, 7,9,12 and 16, an additional 30ml of whole blood will be collected in EDTA tube (lavender top) for flow cytometry of immune cell subsets and 5 ml in serum separator tube (yellow top) for cytokine analysis.

5.8.1 Immune Cell Subsets:

- Effector memory cells (CD 45RA-CCR7-/CD62-)[14]
- Th_{17} cells[15]
- Tregs (CD4+CD25+CD127-)[16]
- TRECS (T cell receptor excision circles)[16]
- CD3+ Cells (T con)[17]
- CD 19+(B cell marker)[1]
- CD3-CD16+CD56+(NK cells)[12]
- FoxP3 gene methylation status

5.8.2 Inflammatory Markers:

- IFN-γ, TNF α[15]
- IL-2,IL-4,IL-6,IL-8[15,20]
- IFN-γ, TNF α[15]
- BAFF[19]
- IL-2 receptor α (IL2Rα) and hepatocyte growth factor (HGF) for systemic GVHD[21]
- Elafin for skin GVHD[22]
- Regenerating islet-derived 3α (REG3α) for gastrointestinal GVHD[21]
- Suppression of tumorigenicity 2 (ST2) for steroid-refractoriness[23]
- CXCL9[24]
- Micro RNA analysis[40]
- Plasma banking to be done for microRNA analysis for cGVHD[43].
- Skin biopsy specimens will be taken at beginning and end of therapy as clinically appropriate.

5.9 Definition of Dose-Limiting Toxicity (DLT)

Not applicable. See section 6.0 for dose and schedule modifications for toxicity.

6.0 Dose Delays/Modifications for Adverse Events

Dose delays and modifications will be made using the following recommendations as described below. Toxicity assessments will be done using the CTEP Version 4.03 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.[33]

6.1 IL-2 Therapy

The dose modifications as described below are required for patients in the first 12 weeks of study and recommended but not mandatory for participants proceeding on extended duration therapy at the end of the 12-week study.

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- Unexpected CTC Grade 3 toxicity: IL-2 will be withheld for unexpected non-hematologic toxicities that are grade 3. Expected IL-2 toxicities are listed in Section 8.1.3 If the toxicity resolves to grade 1 or below within 2 weeks, IL-2 can be restarted at a 50% reduced 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, IL-2 will be discontinued.
- Expected non-hematologic toxicity: IL-2 can be withheld and/or restarted at a 50% reduced dose for less than grade 3 toxicity (e.g. local site reactions, persistent constitutional symptoms) in the interest of patient tolerability and at the discretion of the treating physician.
- Severe (grade-4) 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 a 50% reduced 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. Use of myeloid growth factors and transfusion of platelet and PRBC's will be per standard institutional guidelines and is allowed while patients are on trial.
- 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 12 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.
- Treatment Interruption: Within the first 12 weeks of IL-2 therapy, a ≥6-week interruption will result in the participant being considered unevaluable for response (or lack thereof), unless objective improvement of cGVHD is documented with a shorter course of IL-2.

6.2 Extracorporeal photopheresis:

The incidence of ECP reported side effects is extremely low at less than 0.003% especially in cGVHD patient population[31]. The procedural risks with new third generation ECP devices is even smaller[34]. Many of the complications attributable to ECP are related to vascular access. It would be preferable to use peripheral veins, but patients with cGVHD frequently have poor veins, and alternative access must therefore be used. When central venous catheters are used, complications such as infection, clotting in the catheters, deep venous thrombosis, and vessel stenosis can occur.

For this study, modifications of ECP therapy as described below are required for patients in the first 12 weeks of study. Infection: Of note, infection during ECP+ IL-2 treatment is not considered intervention-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 12 of ECP+ IL-2 therapy will have therapy withheld during active infection. They can be considered for restarting ECP after control of infection, at the discretion of the treating physician. CVP line infection and bacteremia/sepsis requiring line removal will result in temporary stoppage of ECP therapy.

- Severe (grade-4) hematologic toxicity: ECP+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, ECP will be restarted per schedule. If peripheral counts do not improve within 2 weeks, or drop again (ANC<500, Plts<10,000) after restarting ECP + IL-2, it will be discontinued. Use of myeloid growth factors and transfusion of platelet and PRBC's will be per standard institutional guidelines and is allowed while patients are on trial.
- Central Line associated venous thrombosis: If adequate flow volumes can be generated with clot in situ and patient has platelet counts >50,000/mm^3, patients will receive systemic anticoagulation with warfarin, enoxaparin or similar anticoagulant and ECP therapy will be continued per schedule. If, however, platelet counts are less than 50,000/mm^3, precluding anticoagulation therapy, the central line will be removed and therapeutic intervention stopped. ECP will be continued if peripheral access is available.
- Unexpected CTC Grade 3 toxicity: ECP+IL-2 will be withheld for unexpected non-hematologic toxicities that are grade 3. Expected ECP toxicities are listed in Section 8.1.4. If the toxicity resolves to grade 1 or below within 2 weeks, ECP+IL-2 can be restarted If the toxicity does not resolve in 2 weeks to grade 1 or below, or recurs to grade 3 or above after restarting ECP+IL-2 will be discontinued.

7.0 Data and Safety Monitoring, Unanticipated Problems and Adverse Event Reporting

7.1 Definition of Risk Level

This is a Risk Level 3 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. This determination was made because it is a Phase II clinical trial where the risks are at least balanced by the potential benefit to subjects and the importance of the knowledge that may result.

7.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the PI, Collaborating Investigator, CRA, protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of any stopping rules for safety and efficacy.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).

Adverse Events and Serious Adverse Events

The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

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 from day 1 of treatment until 30 days after the last IL-2 injection.
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:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Reporting Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

Serious Adverse Event (SAE) [Modified from the definition of unexpected adverse drug experience in 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy [policy effective date: 05/14/14]. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

Adverse Event Name and Severity

The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.03. GVHD assessments by NIH consensus criteria will also be used.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Adverse Event Attribution

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

Definite - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.

Probable - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).

Possible -The AE may be related to the investigational agent or study procedure and may be related to another cause(s).

Unlikely -The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).

Unrelated -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

Deviations and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11].

Single Subject Exception (SSE)

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11]. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

Unanticipated Problem (UP) – Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy [policy effective date: 05/14/14] using iRIS.

8.0 Agent Information and Risks

8.1 Interleukin-2 (IL-2)

8.1.1 Description

Chemical name: Recombinant human Interleukin 2 /IL-2 (Proleukin®)

Classification: Cytokine

Mechanism of action: Lymphocyte Growth factor.

IL-2 primarily promotes effector T cell responses in vivo, and is now known to be a cytokine critical for the development, expansion, survival and peripheral activity of regulatory T cells (Treg)[13,25].

8.1.2 Toxicology

IL-2 administration by the SC route has been evaluated in several multiple-dose studies in rats for up to 13 weeks of daily. 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[35].

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 animal 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 full prescribing information[36].

Low-dose IL-2, administered s.c. for extended periods, has been evaluated in patients with HIV infection. In one study of ultra-low-dose in 11 patients with HIV infection[37], s.c. IL-2 in doses ranging from 0.4-1.4 million Units /m^2/day, s.c. IL-2 was well absorbed after administration, with a T_{max} of 4.4 ± 1.5 hours and was undetectable 24 hours after injection.

Low-dose IL-2 has also been safely used in patients with hematologic malignancy after allogeneic HSCT. Rizzieri et al.[38] reported use of IL-2 starting six weeks post Allo-HCT using T depleted grafts at dose of 1 million units/m^2. Patients were treated on a four-weeks-on and 2-weeks-off schedule for up to a year to determine if relapse rates were reduced in the setting of non-myeloablative HCT with immune modulation by post HCT IL-2. Fatigue was the main side effect noted with this dose of IL-2. Seven of the 8 patients completed at least 2 cycles, though 1 patient withdrew after cycle 1 and 3 patients withdrew after 2 cycles (3 months) due to excessive fatigue, and 1 withdrew after 6 cycles due to persistent skin GVHD. One further patient had early progressive disease (relapse) after 2 cycles of therapy. Though only 2 completed the maximum of 8 cycles of therapy over the 1 year period, only 1 patient was unable to tolerate at least 2 cycles (3 months) of therapy. Both patients with a mismatched donor tolerated only 2 cycles of therapy, though 1 remains in a long term remission.

In one study by Soiffer et al.[39] , IL-2 was administered by continuous IV infusion to 13 patients with T-cell depleted (TCD) allogeneic HSCT (n=6) and (n=7) patients post autologous HCT. IL-2 was infused at a dose of 0.2 million IU/m^2 /day by hickman catheter for 90 days using a portable pump beginning at a median of 85 days. The majority of patients who received prolonged continuous infusion of low-dose rIL-2 post-BMT tolerated treatment without severe toxicities. No patient developed pulmonary capillary leak syndrome, severe hypotension requiring pressors, oliguria, azotemia, or hyperbilirubinemia during the 90-day course. Two individuals, both recipients of T-depleted allogeneic marrow, developed fever (38.5º C), nausea, and vomiting within 72 hours of initiation of IL-2. When therapy was interrupted, symptoms resolved within 3 days. Fatigue was the most common complaint and occurred in six patients-it did not interfere with daily activities. A 5% to 7% gain in body weight was documented in 5 of 13 individuals, all of whom had peripheral edema. Five patients developed a nonproductive cough; however, only one (with preexisting emphysema) complained of subjective dyspnea on exertion. Cough abated within 1 week of completing treatment in all patients. Localized rashes occurred in six patients, but were transient and resolved before discontinuing IL-2. Immunophenotypic analysis of PBMC was performed routinely while patients received IL-2. The fraction of lymphocytes expressing the NK cell-associated antigen NKHl (CD56) increased steadily over time, rising from a median of 15% initially to 70% within 8 weeks of initiating IL-2 therapy and there was a concomitant increase in the absolute number of NK cells as well.

In DFCI 07-083[13], the safety and immunologic activity of low-dose interleukin-2 in patients with active cGVHD was assessed. The primary objective of this study was to determine the MTD and toxicity profile of an 8 week course of IL-2 in patients with chronic GVHD who had an inadequate response to steroids. Eligibility included patients with cGVHD requiring systemic therapy that had not responded to at least 0.25 mg/kg prednisone for a 4 week period, absence of infection, and stable doses of immune suppression for 4 weeks. The study has a Phase 1 dose escalation design with 3 dose levels (0.3, 1, and 3 x 10^6 IU/m^2/day for 56 days). 29 patients were accrued on protocol; with 28 subjects evaluable for toxicity and 23 evaluable for response. The interval between onset of chronic GVHD and initiation of IL-2 was 803

days (range, 117-2624). Patients were on a median of 3 concurrent agents (range, 1-3) for cGVHD. Skin and fascia were primary sites of involvement, but lung, liver, oral, and ocular sites were involved as well. Dose-level-C (3 x 10^6 IU/m2/day) was infeasible due to persistent constitutional side-effects requiring dose reduction. Dose-level-B (1 x 10^6 IU/m^2/day) was determined to be maximum-tolerated-dose in this patient population. 2 patients developed dose-limiting-toxicity (thrombotic microangiopathy). 3 had CTC grade ≥3 infections unrelated to IL-2. None experienced GVHD flare. There was no malignant disease relapse. 12/23 patients had major responses. These results suggest that low-dose IL-2 can be administered safely in patients with active inflammation of chronic GVHD.

8.1.3 Expected Toxicities with IL-2

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.

Local Reactions (expected in 10% patients): Patients receiving daily SC low-dose IL-2 have been reported to develop injection site reactions, typically focal erythema that resolved in a few days; and induration that resolved after 2-3 weeks. Dose interruptions may occasionally be required in patients with more marked induration (CTCAE 4.0; Grade 3). Lengthy dose interruptions may result in patients being unevaluable for response (Section 6.1)

Constitutional symptoms (expected in 5% patients): In the allo-HCT setting, some patients on low-dose IL-2 developed fever, nausea and arthralgia within 72 hours of starting IL-2. Interruption of therapy for 3 days is recommended if symptoms reported are severe and affecting ADL's i.e. Fatigue ≥ Grade 3 per CTCAE 4.0 criteria. If symptoms of fatigue resolve in 72 hours; IL-2 may be restarted at 50% of prior dose. If however symptoms of fatigue persist and patient is unwilling to continue therapy on protocol, they will be removed from study. Secondary/reversible causes of fatigue, such as sepsis, hypotension, hypothyroidism will be ruled out prior to attributing this to IL-2.

Thyroid dysfunction (expected in 5% patients): Thyroid function test abnormalities were noted in some allo-HCT patients on low-dose IL-2. Only one patient however developed clinical hypothyroidism necessitating therapy while on IL-2[13]. 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.

Hematologic (expected in 5% patients): Early post-HSCT, low-dose IL-2 caused an initial decrease in the absolute lymphocyte count in 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.
- The platelet count decreased by >20% in some allo-HCT 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 changes in monocyte or neutrophil counts were observed. No significant impact of low-dose IL-2 on hemoglobin levels or reticulocyte counts was noted.

Thrombotic Microangiopathy (TMA) (expected in 7% patients): Koreth et al.[13] reported 2 patients on daily SC low-dose IL-2 who 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.

8.1.4 Pharmacology – Handling, Storage, Dispensing and Disposal

8.1.4.1 Form:

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) or subcutaneous (SC) administration. Store vials of lyophilized IL-2 in a refrigerator at 2-8ºC (36-46ºF). Do not use beyond the expiration date printed on the label.

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)

8.1.4.2 Storage and Stability:

Store vials of lyophilized IL-2 in a refrigerator at 2-8ºC (36-46ºF). 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 2-8ºC, 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 2-8ºC (36-46ºF) 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). 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.

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

8.1.4.3 Compatibility:

IL-2 is not given IV, hence compatibility issues with other concomitant IV drugs are not applicable.IL-2 has been used in combination with steroids, mycophenolate mofetil, calcineurin inhibitors, sirolimus and imatinib without any adverse effects.

8.1.4.4 Handling

The single-daily-use syringes containing reconstituted and diluted solutions should be stored in a refrigerator at 2-8ºC. DO NOT FREEZE. The product should be inspected visually for particulate matter or discoloration and brought to room temperature before administration.

8.1.4.5 Preparation:

IL-2 will be reconstituted in 1 ml Becton Dickinson single use syringes. All IL-2 syringes for daily SC outpatient use should be prepared in the pharmacy at the same time, after diluting the 22 MIU IL-2 vial with 1.2 ml SWFI and 4.8 ml D5W (final IL-2 concentration=3.6 MIU/mL), and any remaining product

should be immediately discarded. During reconstitution the SWFI should be directed at the sides of the vial to avoid foaming, and the contents of the vial should be gently swirled. THE VIAL SHOULD NOT BE SHAKEN.

8.1.4.6 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.

8.1.4.7 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.

8.1.4.8 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.

8.2 Extracorporeal Photopheresis

8.2.1 ECP Expected Toxicities (Incidence less than 5%)

Hypotension: Transient hypotension is noted with ECP related to intravascular volume depletion associated with ECP. Vitals signs will be monitored closely per SOP and volume resuscitation with normal saline (500mL over 30 minutes) or 5% albumin will be used per recommendations of Transfusion medicine physician.

Numbness and Tingling: This is related to hypocalcemia related to ECP secondary to anticoagulant. Patients will be given TUMS to swallow and IV calcium gluconate 10mL of 10% solution slow iv over 10 mins to be given in patients not responding to PO calcium supplementation. All patients will have 25 Hydroxycholecalciferol levels checked and patients with vitamin D deficiency will receive replacement therapy with ergocalciferol.

Fatigue: This is noted in patients post ECP session and in setting of refractory cGVHD may be multifactorial and related to underlying condition, chronic steroid use and debilitation. If KPS declines to the point that ADL's cannot be sufficiently performed, patients will continue ECP on protocol. They will be encouraged to be ambulatory and do light exercise as tolerated to combat fatigue.

Central Line Infection: This is related to immunocompromised status of patients with cGVHD and frequent VAD access. Standard antimicrobial prophylaxis and surveillance cultures will be drawn per clinical guidelines. Catheter related blood stream infection will require intravenous antimicrobial therapy and infectious disease service will be consulted as appropriate.

Central Line associated thrombosis: This will be treated with systemic anticoagulation if platelet counts are greater than 50,000/mm ^{3} or line removal if systemic anticoagulation can't be given.

Cytopenias: This is expected from combination therapy with ECP and IL-2. Transfusion and growth factor support per institutional guidelines is permitted. See section 6.2 for detailed management of hematologic toxicities anticipated from ECP.

Tenderness at needle site: This is a common occurrence, but rarely produces more than temporary inconvenience.

Hematoma: Leakage of blood from vein into the surrounding tissues causes bruising. This could occur with any needle puncture into a vein and could produce pain or even limit the full use of the affected arm for a few days. Such a leak can occur at any point or after the treatment. Pressure bandage applied after treatments are intended to limit the occurrence of this complication.

Loss of blood: Leakage/breakage of the plastic tubing rarely occurs but may develop during the treatment. This would result in potential contamination of the portion of blood in the cell separator. As a safety measure, this blood will not be returned to the patient.

9.0 Correlative/Special Studies

9.1 Immune Correlative Studies:

Chronic GVHD is associated with a deficiency of T-regs, NK cells and naive CD8+ T cells. Studies support increased pro-inflammatory cytokines in chronic GVHD cases, including TNF-α, IL-6, IL-1β, IL-8, soluble alpha chain of the IL-2 receptor shed by activated T cells (sIL-2R) and IL-1Rα, among others and decrease in anti-inflammatory cytokines such as IL-10 . Early-onset chronic GVHD had increased soluble B cell activating factor (sBAFF), anti-dsDNA, sIL-2Rα and sCD13. Late-onset chronic GVHD cases had increased sBAFF and anti-dsDNA. Additionally, early onset chronic GVHD demonstrates decreased IFN-γ and IL-2, while late-onset chronic GVHD had decreased IL-4, IL-2 and FOXP3.CRP and C3 and C4 complement levels are also elevated in cGVHD.

The primary immunologic endpoint is a fold-increase in T-reg and the T-reg/-Tcon ratio at 3 months of (ECP+IL2) therapy. Protocol-specified immunophenotypic analyses will be performed at baseline and at 3, 7, 9 & 12 weeks during treatment with ECP +IL-2 and 4 weeks after discontinuation of combination therapy (week 16 for those who complete treatment).

The following analysis will be performed:

9.1.1 Immune Cell Subsets:

- Effector memory cells (CD 45RA-CCR7-/CD62-)[14]
- Th_{17} cells[15]
- Tregs (CD4+CD25+CD127-)[16]
- TRECS (T cell receptor excision circles)[16]
- CD3+ Cells (T con)[17]
- CD 19+(B cell marker)[1]
- CD3-CD16+CD56+(NK cells)[12]
- FoxP3 gene methylation status

9.1.2 Inflammatory Markers:

- IFN-γ, TNF α[15]
- IL-2,IL-4,IL-6,IL-8[15,20]
- IFN-γ, TNF α[15]
- BAFF[19]
- IL-2 receptor α (IL2Rα) and hepatocyte growth factor (HGF) for systemic GVHD[21]
- Elafin for skin GVHD[22]

- Regenerating islet-derived 3α (REG3α) for gastrointestinal GVHD[21]
- Suppression of tumorigenicity 2 (ST2) for steroid-refractoriness[23]
- CXCL9[24]
- Micro RNA analysis[40]

9.2 Methods

9.2.1 Immunologic Assays

Immune cell subset analysis will be performed on peripheral blood mononuclear cells (PBMCs) using the Ficoll density gradient separation. Approximately 15 ml of whole blood will be collected from patients on each visit in EDTA (lavender top) tubes and sent to the research lab for testing. Blood will transferred under a laminar flow hood into a 50mL tube and diluted 1:1 with PBS. The diluted blood will be layered on Ficoll medium by gently pipetting. The 50mL tube will be placed in a swinging bucked centrifuge and spun at 833xg for 20 minutes at 20ºC, acceleration 9 and no brake. 50,000 PBMCs will then be incubated with fluorophore-conjugated monoclonal antibodies: Immunophenotyping will be done for the following markers surface markers (CD 4, CD16, CD 25, CD127, CD45RA, CD62, CD3, CD19 and CD56 to distinguish T cell subsets at baseline and with therapy. Cell analysis will be performed with the use of the FACS Canto II system (BD Biosciences) and FACS Diva software (BD Biosciences).

9.2.2 MicroRNA analysis:

1) Evaluate changes in miR signature in patients with steroid refractory cGVHD on combination therapy with IL-2 and ECP. Experimental Approach: Plasma samples (1 mL) will be collected at baseline (at enrollment) and at 12 weeks (completion of treatment) - from all patients enrolled on protocol. Samples will be stored at -70C freezer. Total RNAs will be extracted from batched samples using 400μL plasma by mirVana PARIS kit (Ambion) according to manufacturer's instructions. *Caenorhabditis elegans* miRNA (cel-miR-39) will be used as spiked in control. Plasma miRNAs will be quantified using SYBR green-based quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). To compare the plasma miR profiles of the two groups of patients, we will use qPCR-based high throughput miRNA array that contains primers specific for 345 well-characterized human miRNAs. Cel-miR-39 will be used for normalization to obtain relative miRNA expression. Statistical Analysis: Hierarchical clustering analysis will be performed using Cluster 3.0.The Mann-Whitney test will be used to compare differences in plasma miR expression between responders and non-responders. Receiver operative characteristic (ROC) curves and area under the ROC curve will be used to assess the sensitivity and specificity of miR biomarkers and logistic regression analysis will be used to develop a miRNA panel predictive of response to therapy. Based on the literature we will focus our analysis on up regulation of miR-146a and down regulation of miR-106b and miR-21.

2) Evaluate miR signature in patients with cGVHD versus matched post HCT controls without cGVHD. Experimental approach. 1 ml plasma sample will be extracted upon enrollment from (n=20) patients with severe steroid refractory cGVHD and a control group of patients without manifestations of cGVHD (n=20) who have similar HCT characteristics (obtained through the Protocol #09050. PI: Nakamura). Plasma samples will be batched and tested for differential miR expression as described in section above-to analyze differential miR expression pattern.

3) Study miRNA expression in Tcon with or without Treg suppression: Experimental approach: Peripheral blood mononuclear cell (PBMC's) from all enrolled patients will be extracted from whole blood using Ficoll paque density gradient centrifugation (GE healthcare) at baseline. The PBMC's will then be flow sorted into 95% pure population of Tregs (CD4+CD25+CD127-) and Tcons (CD4+CD25+CD127-) using FACSAria cell sorter (BD Biosciences). Co-culture of Tregs and Tcons will be done in 96 well round bottom plates (Corning star, Cambridge, MA). Cells will be plated at

density of $1X10^5$/cells/well in RPMI 1640 media (0.2mL/well) and incubated at 37C for 72 hours. Cultures will be stimulated by 1μgm/mL anti-CD3 antibody (BD PharMingen, San Diego, CA). miRNA expression of Treg suppressed and Treg unsuppressed Tcons will be investigated by miRCURY LNA™ microRNA Array (Exiqon, Vedbaek, Denmark). The intensity of signal will be calculated after background subtraction. The threshold value to screen up or down regulation of miRNAs will be a fold change≥2.0 or ≤ 0.5. Analysis Plan: The specific time trend for each cell subset, cytokine and miR will be assessed using linear mixed effects modeling. The overall fit of the model will be evaluated graphically by taking a scatter plot of data for each and applying an overlay of the line generated by the model. An evaluation of the regulation patterns over time will be assessed by examining the slope patterns. To assess the possible impact of changes in expression on T cells, functional regression modeling will be used. Similar modeling techniques will be used to assess the impact of clinical events on expression.

9.2.3 Cytokine assays:

Serum separator tubes will be used (yellow top) will be used to collect samples (5 ml) on each visit. Samples will be allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Serum will be removed aliquotted and stored samples at ≤ -20 °C for batched analysis as described below.

REG3 ELISA kits will be purchased from MBL International (Ab-Match Assembly Human PAP1 kit and Ab-Match Universal kit), and measurements will be performed according to the manufacturer's protocol. Samples (diluted 1:10) and standards will be run in duplicate, absorbance will be measured with a SpectraMax M2 (Molecular Devices), and results calculated with SoftMax Pro Version 5.4 (Molecular Devices). Elafin, IL2R, HGF, TNFR1, and IL-8 ELISAs will performed in duplicate using the quantikine immunoassay (R&D Diosystems). The quantikine assay is a 3.5-4.5 hour solid phase ELISA designed to measure human cytokines (TNF-α, Elafin, IL2R, HGF, IL-2, IL-4,IL-6, IL-8, BAFF, IFN-γ and CXCl-4) in cell culture supernates, serum, and plasma. It contains *E. coli*-derived recombinant human cytokine/protein and antibodies raised against these specific this proteins. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for the pre-specified cytokine is pre-coated onto a microplate. Standards and samples are pipetted into the wells and any cytokine present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for the cytokine is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TNF-α bound in the initial step. The color development is stopped and the intensity of the color is measured.

10.0 Study Calendar

***Only done for patients with pulmonary and cutaneous cGVHD, respectively.**

Procedure/Test	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 16
IL-2 (Daily throughout)		----------												----------
ECP		X2	X2	X2	X2	X1		X1		X1		X1		
Informed consent	X													
Medical history	R	R		R				R		R			R	R
Physical Exam	R	R		R				R		R			R	R
cGVHD Symptom score	X			X				X		X			X	X
Pregnancy test	X													
Pulmonary Function *	X												X	
Dermatologic assessment *	R	R		R				R		R			R	R
Weight	R	R		R				R		R			R	R
Performance Status	X	X		X				X		X			X	X
CBC w/diff,	R	R		R				R		R			R	R
Serum chemistry	R	R		R				R		R			R	R
Immune assessment	R			R				R		R			R	R
Thyroid function/Vitamin D level	X												X	
Steroid dose assessment	X	X		X				X		X			X	X
Adverse event evaluation		X ---------- X												X
Drug Diary		X		X				X		X			X	
QOL self assessment (FACT-BMT Version 4)	X							X					X	
Correlative studies	X			X				X		X			X	X

Patients will undergo standardized cGVHD assessment per NIH guidelines (See Appendix C for forms), at baseline and week 3, 7, 9, 12 and 16 on study. cGVHD response will be assessed per NIH consensus criteria. Of note, oral and ocular sites will not be included in determination of response, as additional topical therapy is permitted for those sites.

X2 Indicates ECP will be administered two times a week **X1** Indicates twice weekly ECP treatment

R Indicates medical exam, blood draw or tests to be done as **routine standard of care.**

Immune assessment This includes monitoring immunoglobulin levels and CMV PCR titers

X Indicates that proposed test, medical evaluation, blood draw or questionnaire is for **research purposes**

11.0 Endpoint Evaluation Criteria/Measurement of Effect

11.1 Primary Endpoint

The primary activity endpoint is cGVHD response, based on the 2014 cGVHD NIH Consensus criteria[41]. Confirmation of CR/PR or SD assessed by NIH Consensus criteria.

11.1.1 Response Definitions:

Complete Response:
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 oral, cutaneous, musculoskeletal and pulmonary systems.

Partial Response:
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. The NIH global severity of chronic GVHD assessment criteria will be used to evaluate the response to therapeutic intervention in one or more involved organs [37]. 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.

Progressive Disease: Organ progression: an absolute increase of at least 25% in the scale used to measure disease manifestations related to cGVHD. 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.

11.2 Secondary Endpoints

Toxicity:
Toxicity will be graded according to the NCI-Common Terminology Criteria for Adverse Events version 4.03[33]. Unacceptable toxicity will be defined as any toxicities that are at least possibly related to either ECP or IL-2 and occur during cycle 1 (within 10 days of initiation of therapy).

Overall Survival:

Defined as the time interval from date of first dose of study drug to date of death from any cause.

Failure-free Survival:

Defined as the time interval from date of first dose of study drug to first documented cGVHD progression (necessitating change of treatment), malignancy relapse or progression or death from any cause, whichever occurs first.

Non-Relapse Mortality:

Defined as time interval from date of first dose of study drug to death from any cause among patients without active disease.

Chronic GVHD Symptom Score:

Patients will self-report symptoms and signs of cGVHD using the validated chronic GVHD Symptom Scale. Self-Reported symptom Scales will be obtained at baseline and weeks 3, 7, 9, 12 and 16.

Steroid Use for Chronic GVHD:

Patients will have their total daily dose of corticosteroids recorded at baseline, and at 3, 7, 9 12 and 16 (or 4 weeks after end of treatment) weeks of IL-2+ ECP therapy. In the case of alternate daily dosing of corticosteroids, the average daily dose will be recorded for study purposes.

Immune Assessment:

Patients will undergo testing for immunologic function, performed at baseline and at weeks 3, 7, 9, 12 and 16 (or 4 weeks after end of treatment). Testing will include: quantitative immune globulins; plasma banking; and storage of additional mononuclear cells.

ECP Performance Parameters: Clinical Response to therapy will be co-related with ECP performance parameters which will include median time spent on ECP machine, second versus third generation device (Cellex vs XTS) and flow rates.

12.0 Data Reporting

12.1 Confidentiality and Storage of Records

This protocol uses Electronic Data Collection, and the data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

12.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

12.1.3 Data Collection Forms and Submission Schedule

All data will be collected on Medidata Rave®. Data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements.

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 10 business days of registration
Baseline Assessment Forms	Within 10 business days of registration
Treatment Forms	Within 10 business days of treatment administration
Adverse Event Report Forms	Within 5 business days, but only through day 60 (DLT period)
Response Assessment Forms	Within 10 business days of response assessment
Other assessment forms (e.g. concomitant meds, chemistry, hematology, neuro exam, physical exam)	Within 10 business days of the assessment
Off Treatment/Off Study Forms	Within 10 business days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 10 business days of the protocol defined follow up visit date or call

13.0 Statistical Considerations

13.1 Study Design, Sample Size, and Accrual Rate

This single center/single arm, phase II clinical trial is designed to evaluate the anti-cGVHD activity of extracorporeal photopheresis (ECP) when combined with low dose IL-2, in patients with steroid refractory cGVHD, as assessed by overall cGVHD response rate (CR+PR+SD). The primary endpoint overall response (CR+PR+SD) is based on the NIH cGVHD consensus criteria; calculated as the percent of evaluable patients that have confirmed CR/PR or SD

This study will implement a Simon, optimal, two-stage design to evaluate the anti-cGVHD activity of the ECP/IL-2 regimen. The trial is expected to enroll 24 patients e sample size is based on the desire to discriminate a promising response rate of 50% from a current response rate of 25%. The null hypothesis

that the true response rate is 25% will be tested against a one-sided alternative. In the first stage, 9patients will be accrued. If there are 2 or fewer responses in these 9 patients, the study will be stopped. Otherwise, 15 additional patients will be accrued for a total of 24. The null hypothesis will be rejected if 10 or more responses are observed in 24 patients. This design yields a type I error rate of 0.05 and power of .80 when the true response rate is 50%. Currently, about 20-25 new patients are referred to ECP per year. We expect that 6 patients to be enrolled per year with the expected completion of accrual in 4years.

Evaluable for Response: As part of the primary analysis, patients will be considered evaluable for response if they are eligible, have baseline disease assessments, and receive protocol treatment of any duration. All such patients will be evaluated for clinical response.. Our primary outcome will be the clinical response rate measured in all patients who are enrolled and receive any amount of protocol therapy.

Patients who are consented for the study but withdraw for any reason (progression of GVHD, infectious complications, insurance denial etc) prior to initiation of protocol therapy will not be eligible for response evaluation.

Evaluable for Toxicity: Patients will be considered evaluable for toxicity if they receive any study drug.

Safety Analysis and Stopping Rules for Excessive Toxicity

The following table will be consulted as relevant toxicities are encountered. The early stopping rule for safety/toxicity will be assessed for each patient after 3 weeks of treatment. The expected rate of unacceptable toxicity should not be ≥33%. See the table below for detailed early stopping rules. These rules are in addition to the quarterly review of all toxicities submitted to the COH DSMC. Patients with ongoing toxicity (toxicity persisting beyond day +21) will be followed until resolution or stability. If more than the specified number of patients has significant treatment related toxicities, patient accrual will be halted and a full review of the data by the Data Safety Monitoring Committee (DSMC) will be mandated. Patient accrual will not resume until approved by the DSMC to do so.

# of patients treated at phase II dose	# of patients with unacceptable toxicity to halt enrollment[1]	# of patients expired due to treatment related causes that would stop the study[2]	Given the following toxicity rates, cumulative probability of early stopping:		
			15%	33%	45%
6	2	2	0.22	0.64	0.84
9	3	3	0.25	0.72	0.89
12	4	4	0.26	0.75	0.93
18	6	6	0.27	0.80	0.95

[1]: For each unacceptable toxicity, halt enrollment and evaluate if the cumulative # of patients reaches or exceeds the specified limits.

[2]: Expected treatment related mortality should not exceed 33%.

In addition, cumulative safety data will be reviewed on a quarterly basis in order to identify safety concerns that may emerge due to cumulative exposure.

13.2 Statistical Analysis Plan

Patient demographic and baseline characteristics, including age, gender, medical history, and prior cGVHD therapy, will be summarized using descriptive statistics. For continuous variables, descriptive

statistics (number [n], mean, standard deviation, standard error, median (range) will be provided. For categorical variables, patient counts and percentages will be provided.

The overall response rate (CR+PR) will be calculated as the percent of evaluable patients; exact 95% confidence intervals will be calculated for these estimates. Response rates will also be evaluated based on number and type of prior therapy(ies). Time to response, duration of response, and survival will be estimated using the product-limit method of Kaplan and Meier.

Observed toxicities will be summarized in terms of type (organ affected or laboratory determination), severity, time of onset, duration, probable association with the study treatment and reversibility or outcome. Baseline information (e.g. the extent/type of prior therapy) and demographic information will be presented as well to describe the patients treated in this study.

14.0 Human Subject Issues

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2 Recruitment of Subjects:

Subjects will be recruited from City of Hope

14.3 Advertisements

Although none are planned, advertisements to include print, media (radio, television, billboards), telephone scripts, lay summary to be posted on City of Hope's public Clinical Trials On-Line^{SM} website, etc., will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

14.4 Study location and Performance Sites

This study will be performed at COH.

14.5 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI).

The study will record individual immunological, clinical response and side effect profile to IL-2 and ECP combination therapy and the information collected will be linked to the subject's identity using a coded study number. In addition, results of screening tests to rule out HIV, HBC, and HCV performed in the Department of Transfusion Medicine will be used with the participant's permission if performed in the past 4 months. The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

14.6 Financial Obligations and Compensation

Cost for IL-2 and ECP will be covered by third party payers the enrolled subjects will not bear the cost of treatment. The cost for immunologic studies will be covered by in house funding support. Intramural funding will also be used to cover cost of personnel and biostatistical analysis of data generated from this clinical trial. Our past experience suggests that insurance coverage rate is 60%. Patients who sign consent but are not eligible for IL-2 in case insurance is unwilling to pay for IL-2 will be deemed as screen failures

and will not be eligible for final evaluation. They will continue regular follow up and standard of care treatment.

The standard of care drug(s) and procedures provided will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

14.7 Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be provided. Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education and proctoring will be ineligible for enrollment. For those subjects who do comprehend the fundamental aspects of the study, consent will be obtained and documented, followed by eligibility testing. The research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment. No subjects will be enrolled into the study without first demonstrating full comprehension of the study and their rights as research subjects.

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