Clinical Trial Protocol

LOW DOSE IL-2 FOR THE TREATMENT OF ULCERATIVE COLITIS

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3 PROTOCOL SYNOPSIS.

3.1 Study Title.

A Phase I Study of Low Dose Subcutaneous Interleukin-2 (IL-2) For The Treatment of Ulcerative Colitis.

3.2 Objective.

To determine the safety and maximum tolerated dose of daily subcutaneous IL-2 in the treatment of moderate-to-severe ulcerative colitis (UC).

3.3 Subject Eligibility.

- Adult subjects with moderate-to-severe UC (Mayo score 6-12).
- Declined, or failed to tolerate or respond to a standard course of UC medication prescribed with the intention of inducing or maintaining remission.

3.4 Registration Contacts.

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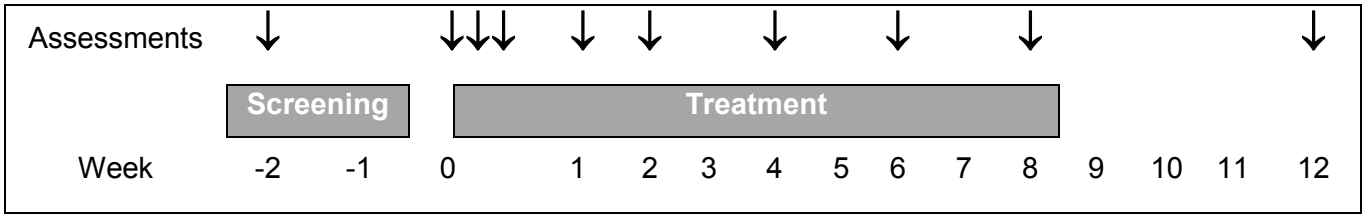
3.5 Schema.



3.6 Evaluation.

- Assessment of study drug toxicity.
- Assessment of UC disease activity and mucosal healing.

4 BACKGROUND AND SIGNIFICANCE.

4.1 The Current Requirement for Novel Therapies in Ulcerative Colitis.

Ulcerative Colitis (UC) is a chronic inflammatory disease of the rectum and colon, causing bloody diarrhea, urgency and tenesmus[1]. UC has a population-based incidence of 14/100,000, with a prevalence of 240/100,000, affecting an estimated 750,000 people in the United States[2,3]. Mucosal inflammation in UC is managed with anti-inflammatory and immune-modulating drugs to induce remission (e.g. mesalamine, corticosteroids, biologic agents) and, in patients with frequent flares in disease activity, immune-modulating drugs to maintain remission (e.g. azathioprine, 6-mercaptopurine [6-MP], methotrexate, biologic agents). A substantial proportion of patients have sub-optimal responses to current therapies or develop adverse events. Furthermore, surgical resection of the colon and rectum is the only currently available "curative" treatment for those with refractory disease. However, surgery is unpopular amongst patients and is associated with its own spectrum of complications. Consequently, an urgent need exists to identify new, more effective therapies for UC with more acceptable safety profiles.

A significant unmet need persists for new approaches to therapy as existing medications have limited benefit in UC. The results of clinical trials to induce remission in UC are surprisingly modest. In mild-to-moderate UC, mesalamine induces remission in approximately 40% of patients[4]. As much as a third of newly-diagnosed UC patients will have a need for oral or parenteral corticosteroids to induce remission[5]. In these patients, corticosteroids induce remission in over half, with a partial response in an additional third, but at one year 28% of those initially treated with steroids are steroid-dependent and a third require surgery[5]. The key finding of Truelove & Witts' 1955 study of cortisone in UC was that steroids reduced mortality in this previously fatal disease[6]. In patients with moderate-to-severe UC, 69% respond to infliximab, with half of those entering remission, but 31% fail to respond[7]. Many who initially respond to the anti-TNFα medications now indicated for UC (infliximab, adalimumab or golimumab) will loose response over time. Longer term use of immune modulating drugs to maintain remission is also not without risks, with clinical effectiveness always being balanced against untoward side effects, including anemia, pancreatitis, lymphoproliferative disease and infection[8,9]. Finally, a number of novel agents targeting aspects of the mucosal immune system, such as vitulizumab[10] (an anti-CD3 monoclonal antibody), or secukinumab[11] (an anti-IL-17A monoclonal antibody) have not yielded promising results in recent clinical trials in IBD.

Colectomy rates reflect a failure of medical management of UC and an unmet therapeutic need. Six percent undergo colectomy within a year of diagnosis[12]. In patients with moderate to severe colitis, requiring corticosteroid treatment at presentation, this figure rises to 29%[5]. Overall, 24% will have had a colectomy by the 10th year of disease[13]. Despite a variety of new medications, the rate of colectomy in UC has not substantially changed in the last five decades[14,15], indicating that current medicines have failed to alter the natural history of UC.

4.2 Regulatory T Cells and Mucosal Inflammation in UC.

Gut mucosal homeostasis is characterized by a constant interaction between commensal intestinal microbiota and the mucosal immune system. In order to prevent inappropriate inflammation to commensal bacteria in the gut, a population of thymus-derived regulatory T cells (termed "natural" T_{regs}) characterized by $CD4^+CD25^+FOXP3^+$ expression in mice and $CD4^+CD25^{hi}CD127^{lo}FOXP3^+$ expression in humans allows tolerance to "self-antigens"[16]. Activation of T_{regs} by inflammatory stimuli causes them to antagonize the activation and effector function of a range of pro-inflammatory immune cells that are contemporaneously activated by similar stimuli. In humans, this includes reduced proliferation and cytokine production by responder $CD4^+$, $CD8^+$ and $\gamma\delta$ T cells, and modulation of antigen presenting cells (APC) and natural killer (NK) cell responses[17-20]. Furthermore, T_{regs} are effective at treating murine intestinal inflammation *in vivo*. By antagonizing activation of conventional effector $CD4^+CD25^{lo\text{-}int}$ T cells (T_{con}), T_{regs} both prevent the onset of colitis[21,22] and treat established colitis[23] in the T_{con} mediated $CD4^+CD45RB^{hi}$ adoptive transfer model of colitis in mice. T_{regs} also treat colitis mediated by the innate immune system in both the RAG2$^{-/-}$ 129/SvEv *Helicobacter hepaticus*[24] and TRUC (T-bet$^{-/-}$ x RAG2$^{-/-}$ UC)[25] mouse models of colitis.

The consequences of defective T_{regs} can be best appreciated in the X-linked *Foxp3* mutant *scurfy* mouse, where hemizygous males develop lethal multi-organ autoimmunity, including enteritis, due to $CD4^+$ hyperactivation[26]. In humans, *FOXP3* mutations are associated with the IPEX syndrome (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked), which causes gastrointestinal inflammation due to dysregulated lymphocyte proliferation[27].

T_{reg} defects are also seen in Wiskott-Aldrich syndrome, an X-linked primary human immunodeficiency characterized by recurrent infections, thrombocytopenia, eczema and autoimmunity[28]. Our laboratory and others identified that defective expression of Wiskott-Aldrich syndrome protein (WASP) results in decreased T_{reg} numbers *in vivo* and decreased proliferation

and expression of IL-10 and TGFβ *in vitro*[28-30]. WASP$^{-/-}$ Tregs are also unable to prevent CD45RB^{hi} colitis in mice [28]. Interestingly, WASP deficiency in the innate immune system also impairs the ability of adoptively transferred wild type (WT) T_{regs} to suppress naïve T cell-mediated colitis, although this can be overcome by increased T_{reg} dose[31].

WASP$^{-/-}$ T_{regs} are defective in IL-10 secretion[28]. We also recently identified causative interleukin-10 receptor (IL-10R) polymorphisms responsible for the development of infantile IBD that, at least in part, may be associated with defective T_{reg} function[32,33]. In this regard, ablation of IL-10R in murine T_{regs} impairs STAT3-dependent suppression of IL-17-mediated colitis[34]. These observations support a role for T_{regs} in preventing inappropriate intestinal inflammation in humans.

In humans in health, CD4$^+$CD25^{hi}CD127^{lo}FOXP3$^+$ T_{regs} comprise 5-10% of peripheral blood (PB) CD4$^+$ lymphocytes and 10-15% of *lamina propria* (LP) CD4$^+$ lymphocytes. The PB T_{reg} fraction is decreased in acute (diverticulitis) and chronic (UC) mucosal inflammation, while colonic LP T_{regs} are increased in active IBD[35,36]. The frequency of PB and LP T_{regs} normalizes in both quiescent and treated UC[35,36], consistent with the hypothesis that T_{regs} migrate and/or are induced *de novo* in the setting of inflammation.

4.3 The Rationale for High Dose IL-2 as a Cancer Therapy: Induction of Effector Immune Cells to Enhance Anti-Tumor Immunity.

IL-2 is a four-bundle α-helical cytokine secreted by many cell types but is primarily secreted by CD4$^+$ lymphocytes in secondary lymphoid organs under steady-state conditions and is strongly induced on antigen-specific activation[37]. At high concentrations, IL-2 activates multiple cell types, inducing T_{con} proliferation and Th1 polarization via STAT5-dependent expression of IL-12Rβ2 and T-bet[38], Th2 polarization via STAT5-dependent early expression of IL-4Rα and chromatin remodelling at the Il-4 locus[39]. It is also responsible for NK cell activation and proliferation[37], and helping primary and secondary expansion of CD8$^+$ T cells[37].

Intravenous (IV) and subcutaneous (SC) IL-2 is currently licensed for the treatment of metastatic melanoma and metastatic renal cell carcinoma. At the high doses of IL-2 approved for the management of these malignancies, IL-2 induces T_{cons}, cytotoxic CD8$^+$ T cells and NK cells[37], with the intention of boosting anti-cancer immunity. Approximately 15% of patients

develop a clinical response[40]. IL-2-mediated immune activation also leads to secretion of TNFα, IFNα and IL-15, which is associated with side-effects[40].

Common side-effects of high dose IL-2 therapy in the treatment for metastatic melanoma or metastatic RCC include: flu-like symptoms, diarrhea, eosinophilia, capillary leak syndrome, impairment of neutrophil function and risk of Gram positive infection and sepsis[40]. Early studies reported treatment-related mortality of 4%, primarily related to a sepsis-like syndrome[40]. Kammula and colleagues treated 1241 patients with IL-2 at the National Cancer Institute between 1985 and 1997[41]. They noted a significant decrease in Grade 3/4 toxicity over time. Comparing the first and last cohorts of 155 patients, serious diarrhea decreased from 92% to 12%, hypotension from 81% to 31%, neuropsychiatric toxicity from 19% to 8%, line sepsis from 18% to 4%, tracheal intubations from 12% to 3%, and cardiac ischemia from 3% to 0%[41]. Treatment-related deaths were not seen in the final 809 patients. The authors attributed the improvement in safety profile to judicious pre-treatment screening, patient optimization and improved recognition and management of treatment-related toxicities.

4.4 The Rationale for Low Dose IL-2 in UC: Selective Induction of Regulatory T Cells to Modulate Immune Responses.

IL-2 signals through a dimeric receptor composed of CD122 (IL-2Rβ) and the γ_c chain (IL-2Rγ)[42]. $CD4^+CD25^{hi}$ T_{regs} constitutively express CD25 (IL-2Rα), a non-signaling molecule that readily associates with the dimeric IL-2 receptor to form a trimeric receptor complex. The trimeric IL-2R, involving CD25, has a 10-100-fold higher affinity for IL-2, compared to the dimeric IL-2R, without CD25[37,43]. In contrast, $CD4^+CD25^{lo\text{-}int}$ T_{cons} constitutively express the dimeric IL-2R and only transiently express CD25 on activation[42]. This confers distinct IL-2 response profiles to $CD4^+CD25^{hi}$ T_{regs} and $CD4^+CD25^{lo\text{-}int}$ T_{cons} in man. Our collaborators have shown that *in vitro* treatment of human PB $CD4^+$ lymphocytes with low-dose IL-2 (1-10 IU/ml) results in STAT5 phosphorylation and proliferation in T_{regs} but not T_{cons}[43]. This differential response is lost *in vitro* at higher IL-2 concentrations (>100 IU/ml)[43,44], consistent with observations that IL-2 expands both T_{regs} and T_{cons} *in vivo* in man, at the doses used for anti-cancer therapy[44,45]. This may explain the limitation of IL-2 in cancer therapy, as T_{reg} generation may limit anti-cancer immunity.

Constitutive expression of CD25 is important for T_{reg} biology, as IL-2 has a non-redundant function in promoting peripheral tolerance *in vivo*[46,47]. Genetic absence of

components of the IL-2 pathway, such as *Il2*, *Il2ra*, *Il2rb* or *Stat5a*, or neutralization of IL-2 results in impaired T_{reg} development, survival and function, leading to lymphoproliferation and autoimmunity in mouse models[37].

4.5 Preclinical and Clinical Studies of Low Dose IL-2 to Selectively Expand Regulatory T Cells *In Vivo* in Man.

Because T_{regs} respond to much lower doses of IL-2 than pro-inflammatory immune cells, "low-dose" IL-2 (some 100-fold lower than that used in anti-cancer therapy) is a conceptually attractive approach to selectively expanding T_{regs} in order to enhance their immune modulating effects in diseases characterized by unopposed immune activation.

In mouse models, low-dose IL-2 prevents graft *vs.* host disease (GvHD) following HLA-mismatched allogeneic bone marrow transplantation (BMT) by selectively expanding T_{regs} from co-administered syngeneic T cell-depleted marrow[45,48]. Low-dose IL-2 also induces remission of autoimmune diabetes in NOD mice by selectively expanding T_{regs} and reducing pancreatic inflammation[48,49]; clinical trials of low-dose IL-2 for type 1 diabetes are currently recruiting or complete (ClinicalTrials.gov NCT01353833, NCT01827735). Stallmach and colleagues showed that a fusion protein of IL-2 bound to IgG2b (IL-2IgG2b) prevents and ameliorates 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice[49,50]. It was subsequently found that IL-2IgG2b caused T_{reg} expansion. We have generated preliminary data showing that TNBS colitis in "humanized" NOD-Scid-$\gamma_c^{-/-}$ (NSG) mice, engrafted with human PB mononuclear cells (PBMCs), can be both prevented and ameliorated by low-dose human IL-2 (Goettel, Snapper, unpublished).

Low-dose IL-2 has been shown to be both safe and effective in the treatment of GvHD. Some years ago, our collaborators safely administered low-dose IL-2 ($0.2\text{-}0.6\text{x}10^6$ IU/m^2/day IV) to 29 patients who underwent CD6$^+$-depleted allogeneic BMT following hematological malignancy, without GvHD, expanding both NK cells (for a graft *vs.* leukemia effect) and T_{regs} (preventing GvHD) *in vivo*, leading to improved disease-free survival[50,51]. Recently, our collaborators showed that low-dose IL-2 ($0.3\text{-}3\text{x}10^6$ IU/m^2/day SC) was safe and effective at treating steroid-resistant chronic GvHD following allogeneic hematopoietic stem cell transplantation (HSCT)[43,51]. Low-dose IL-2 therapy was associated with a sustained increase in circulating T_{regs} and a rapid reduction in T_{con} activation, which correlated with clinical response[43,51].

Low-dose IL-2 is also safe and effective in the treatment of HCV-associated vasculitis and safe in HIV. Saadoun and colleagues used low-dose IL-2 ($1.5x10^6$ IU/day SC) to manage treatment-resistant HCV-associated vasculitis, resulting in selective T_{reg} expansion with a reduction in marginal zone B cells and cryoglobuliemia *in vivo* without increased HCV viremia[52]. Low-dose IL-2 has also been safely used with the intention of boosting $CD4^+$ T cells in HIV, primarily resulting in increased circulating T_{regs}[53].

4.6 Safety Profile of Low Dose IL-2: Side-Effects Seen in Recent Clinical Trials.

Soiffer *et al.*[50] administered low-dose IL-2 by continuous IV infusion to 29 patients who underwent CD6-depleted BMT at least 6 weeks previously, with the intention of boosting Graft *vs.* Leukemia activity. Doses ranged from 0.2 to $0.6x10^6$ IU/m^2/day. 25/29 patients completed 4 weeks of therapy, while 17/29 patients completed at least 8 weeks of therapy. Observed toxicities included fatigue (52%), temperature >38°C (48%), nausea/vomiting (38%), myalgias (31%), diarrhea (24%), edema/weight gain (21%), dyspnea (21%), catheter infection (17%), thyroid dysfunction (7%), thrombocytopenia (<20,000; 7%) and neutropenia (<500; 3%). Toxicities were most prominent in patients on higher doses.

In contrast to IV administration, SC administration is associated with slower absorption with maximum concentrations occurring 2-5h after dosing, 20-30-fold lower peak serum levels and a longer elimination half-life (5h SC *vs.* 1.5h IV)[40], all of which may contribute to a favorable toxicity profile with SC administration.

Koreth *et al.*[51] administered daily SC low-dose IL-2 to 29 patients (28 were evaluable) with steroid-resistant chronic GvHD following HSCT. The maximum tolerated dose (MTD) in this study was found to be $1x10^6$ IU/m^2/day. The higher dose of $3x10^6$ IU/m^2/day was associated with persistent grade 1 constitutional symptoms, necessitating dose reduction. Toxicities related to IL-2 included reversible induration of SC injection sites (n=3/28), grade 2 constitutional symptoms (fever, malaise and fatigue in 1/28), grade 2 renal dysfunction (1/28) and grade 2 thrombocytopenia (1/28). Two patients in this study developed thrombotic microangiopathy-associated renal failure requiring dialysis. Both of these patients received sirolimus and tacrolimus, which are associated with thrombotic microangiopathy. Three patients developed grade 3 or grade 4 infections. One patient with a background of MRSA pneumonia developed recurrent MRSA pneumonia following 7 weeks of therapy. A second patient developed

Hemophilus influenzae type-B bacteremia following 4 weeks of treatment. A third patient had a pre-existing MRSA furuncle, necessitating discontinuation of treatment at day 2.

While the study design of this proposed phase Ib/IIa clinical trial is based on Koreth *et al*'s study, patients with active UC are likely to be fitter than patients with steroid-resistant GvHD following BMT. In a recent study with a more comparable group, Saadoun *et al.*[52] treated 10 patients with HCV-associated vasculitis that was unresponsive to conventional anti-viral therapy and/or rituximab with low-dose IL-2: 1.5 MIU/day for 5 days, followed by three 5-day courses of 3 MIU/day at weeks 3, 6 and 9. Only mild (grade 1) toxicities were observed, including fatigue (4/10), flu-like symptoms (4/10), SC injection site reaction (2/10), arterial hypertension (1/10) and myalgia (1/10).

4.7 Hypothesis.

Based on these data, we hypothesize that low-dose IL-2 will safely expand PB and LP Tregs *in vivo* in patients with UC and be efficacious in controlling disease.

5 STUDY DESIGN AND OBJECTIVES.

5.1 Study Design.

An open-label, phase Ib/IIa clinical trial, with a 3+3 dose escalation design, to determine the safety and the maximum tolerated dose (MTD) of daily SC recombinant human interleukin-2 (IL-2, aldesleukin, Proleukin®) in adult subjects with moderate to severe UC.

5.2 Primary Objectives.

(i). To determine the safety of daily SC low dose IL-2 in subjects with moderate to severe UC

(ii). To determine the MTD of daily SC low dose IL-2 in subjects with moderate to severe UC.

5.3 Secondary Objectives.

(i). To determine the feasibility of administering an eight week course of daily SC low dose IL-2 to subjects with moderate-to-severe UC.

(ii). To determine the clinical response to the above treatment.

(iii). To assess the immunological response to the above treatment, with particular reference to numbers and function of T_{regs} in the blood and intestinal lamina propria (LP)

6 SUBJECT SELECTION.

6.1 Inclusion Criteria.

(i). Age 18-70 years. Maximum age limit for subjects recruited at BCH will be 30 years.

(ii). A diagnosis of UC made by standard clinical, radiological, endoscopic and histological criteria.

(iii). Moderate to severe UC with a Mayo score of 6-12.

(iv). Failure to tolerate or failure to respond to at least one conventional therapy with the intention of inducing or maintaining remission (examples include oral corticosteroids, oral 5-aminosalicylates, azathioprine and/or 6-mercaptopurine, or a TNF antagonist). Corticosteroid dependency (inability to taper oral corticosteroids without a recurrence of disease activity) is also included in this category.

(v). Stable doses of concomitant medications, as defined in Section 7 (page 19).

(vi). A negative pregnancy test in the 2 weeks prior to anticipated commencement of the study drug, in female subjects of child-bearing age. Men and women of reproductive potential must agree to use an acceptable method of birth control during treatment and for six months after completion of treatment.

(vii). Ability to provide informed consent.

6.2 Exclusion Criteria.

(i). A diagnosis of Crohn's disease or indeterminate colitis.

(ii). Requirement for immediate surgical, endoscopic or radiological intervention for toxic megacolon, massive hemorrhage, perforation, sepsis, or intra-abdominal or perianal abscess.

(iii). Ileostomy, proctocolectomy or subtotal colectomy with ileorectal anastomosis.

(iv). History of colorectal cancer or dysplasia.

(v). Positive stool test for *Clostridium difficile*.

(vi). Current medically significant infection.

(vii). Significant laboratory abnormalities;

a. Hb < 8.0 g/dL, WBC < 2.5 x $10^3/mm^3$, Plt < 100 x $10^3/mm^3$.

b. Creatinine ≥ 1.5x institutional ULN.

c. Total bilirubin > 2.0 mg/dL, ALT > 2x institutional ULN. Elevated unconjugated bilirubin related to Gilbert's syndrome is allowed.

d. Abnormal thyroid function tests.

(viii). Positive serology for HIV, hepatitis B virus (HBV) or hepatitis C virus (HCV).

(ix). Positive screening test for tuberculosis (TB).

(x). First dose of an anti-TNFα medication within 4 weeks of anticipated study commencement, or a subsequent dose within 2 weeks of commencement; or ciclosporin or tacrolimus within 2 weeks of anticipated study commencement.

(xi). Received another IND within 5 half-lives of that agent before the planned commencement of SC IL-2.

(xii). Malignancy within the last 5 years.

(xiii). Allergy to any component of the study drug.

(xiv). Pregnant or lactating women.

(xv). Inability to comply with the study protocol or inability to give informed consent.

(xvi). Prior exposure to IL-2.

(xvii). Uncontrolled cardiac angina or symptomatic congestive cardiac failure (NYHA Class III or IV).

7 RULES FOR CONCOMITANT IBD MEDICATIONS.

(i). Subjects receiving oral 5-aminosalycilates or oral corticosteroids (prednisone ≤40mg per day or its equivalent) must receive stable doses for at least 2 weeks prior to commencement of the study.

(ii). Subjects receiving azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, tacrolimus, sirolimus or mycophenolate mofetil must receive stable doses for at least 4 weeks prior to commencement of the study.

(iii). Subjects receiving concurrent tacrolimus and sirolimus will be excluded, due to the increased risk of renal failure seen in previous studies when these medications were co-prescribed.

(iv). Rectally administered steroids or mesalamine will be discontinued 2 weeks before screening.

(v). Subjects receiving IV steroids or biologic agents must undergo a 4-week wash-out period prior to enrolment.

8 SUBJECT ENTRY.

8.1 Recruitment and Accrual.

Subjects enrolled in the trial will be identified through the gastroenterology department at Boston Children's Hospital.

8.2 Study Site Responsible Investigator.

Boston Children's Hospital

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9 TREATMENT PROGRAM.

9.1 Description of Study Design.

This is an open-label phase Ib/IIa clinical trial to determine the safety and maximum tolerated dose of daily SC IL-2 in eligible subjects with moderate to severe ulcerative colitis (UC). Subject cohorts will receive escalating dose levels of IL-2, as shown in Table 1 (page 22). A minimum of three subjects will be treated in each cohort, with an additional ten subjects treated at the presumptive MTD.

9.2 Dose Cohorts.

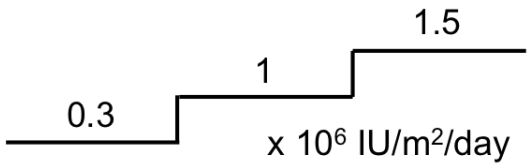
Koreth *et al.*[51] treated subjects with steroid-resistant GvHD with doses ranging from 0.3-3.0x10^6 IU/m^2/day. Doses of 0.3 and 1.0x10^6 IU/m^2/day were well-tolerated. 1.0x10^6 IU/m^2/day was found to be the MTD in this study. Dose limiting toxicity (DLT) occurred at 3.0x10^6 IU/m^2/day. Saadoun *et al.*[52] treated subjects with treatment-resistant HCV-associated vasculitis at a dose of 1.5x10^6 IU/day, which was well-tolerated in this study. The side-effects seen in these studies are detailed in Section 4.6 (page 13).

Dose level A: 0.3x10^6 IU/m^2/day. Koreth *et al.*[51] found that a dose of 0.3x10^6 IU/m^2/day was well tolerated in a cohort of subjects with steroid-resistant GvHD. Consequently, we propose to commence treatment at this dose.

Dose level B: 1x10^6 IU/m^2/day. Provided dose level A is tolerated, the dose will then be escalated to dose level B. This was the MTD in the Koreth *et al.* study[51].

Dose level C: 1.5x10^6 IU/m^2/day. Intolerable constitutional symptoms occurred at the highest dose in the Koreth *et al.* study[51] (3x10^6 IU/m^2/day). Consequently, we propose that the highest dose in this study will be 1.5x10^6 IU/m^2/day, or 50% of the dose at which intolerable constitutional symptoms occurred in that study. This dose has been included to maximize the potential for clinical response, while minimizing the potential for toxicity. This dose remains two orders of magnitude below those used in studies of IL-2 for metastatic melanoma and RCC[40].

Table 1: Dose Escalation Cohorts.



Cohort	IL-2 Dose ($IU/m^2/day$)	Projected n in each cohort
Dose Level A	0.3×10^6	3
Dose Level B	1×10^6	3
Dose Level C	1.5×10^6	3 + 3 + 10*

*Assuming dose level C is shown to be the MTD.

Adapted from Koreth *et al*.[51]

9.3 Dose Escalation Schema.

There will be 3 dose levels, ranging from 0.3 to 1.5×10^6 $IU/m^2/day$. Each subject will be treated at a single dose level. The first three subjects will be enrolled at the lowest dose level: dose level A. These subjects will be treated sequentially, so that none of these subjects will receive the study drug concurrently. The dose will be escalated as per the protocol below.

The maximum tolerated dose (MTD) is the highest tolerated dose level at which a minimum of 6 subjects have been evaluated, with fewer than 2 evaluable subjects in 6 experiencing a dose limiting toxicity (DLT); i.e. DLT in ≤1/6 evaluable subjects.

A dose escalation schema has been designed in order to identify the MTD.

9.3.1 Dose Level A.

Three evaluable subjects will be recruited at dose level A. They will be monitored for the development of DLT during their 8-week treatment course.

If none (0/3) of these subjects experience a DLT during the 8-week treatment, dose escalation will occur so that the next three subjects will be enrolled at dose level B.

If one of the first three subjects treated (1/3) at dose level A experiences a DLT during the 8-week treatment, an additional 3 subjects will be enrolled at dose level A. Up to six subjects in total will then complete treatment at dose level A. If no further DLTs occur in this group of 6 subjects (i.e. 1/6 subjects with DLT), dose escalation will occur so that the next three subjects will be enrolled at dose level B. If DLTs occur in two subjects in this group (i.e. 2/6 subjects with DLT), then dose level A has not been tolerated and the study will be terminated early.

If two of the first three subjects treated (2/3) at dose level A experience DLT during the 8-week treatment, then dose level A has not been tolerated and the study will be terminated early.

The dose may be reduced from dose level B to dose level A. If this occurs and six subjects have already been treated at dose level A, then dose level A is the MTD. If this occurs and only three subjects have been treated at dose level A, then a further three subjects will be recruited at dose level A. If 0 or 1 subject in this group of 6 experience a DLT, then dose level A will be the MTD. If 2 subjects in this group of 6 experience a DLT, then the study will be terminated early.

9.3.2 Dose Level B.

After successful dose escalation from dose level A, the next three evaluable subjects will be treated at dose level B. They will be monitored for the development of DLT during their 8-week treatment course.

If none (0/3) of these subjects experience a DLT during the 8-week treatment, dose escalation will occur so that the next three subjects will be enrolled at dose level C.

If one of the first three subjects treated at dose level B (1/3) experiences a DLT during the 8-week treatment, an additional 3 subjects will be enrolled at dose level B. Up to six subjects in total will then complete treatment at dose level B. If no further DLTs occur in this group of 6 subjects (i.e. 1/6 subjects with DLT), dose escalation will occur so that the next three subjects will be enrolled at dose level C. If DLTs occur in two subjects in this group (i.e. 2/6 subjects with DLT), then dose level B has not been tolerated and the dose will be reduced to dose level A (see Section 9.3.1, page 22).

If two (2/3) of these subjects experience a DLT during the 8-week treatment, then dose level B has not been tolerated and the dose will be reduced to dose level A (see Section 9.3.1, page 22).

The dose may be reduced from dose level C to dose level B. If this occurs and six subjects have already been treated at dose level B, then dose level B is the MTD. If this occurs and only three subjects have been treated at dose level B, then a further three subjects will be recruited at dose level B. If 0 or 1 subject in this group of 6 experience a DLT, then dose level B will be the MTD. If 2 subjects in this group of 6 experience a DLT, then the dose will be reduced from dose level B to dose level A.

9.3.3 Dose Level C.

After successful dose escalation from dose level B, the next three evaluable subjects will be treated at dose level C. They will be monitored for the development of DLT during their 8-week treatment course.

If none or one (0-1/3) of the first three subjects recruited at dose level C experience a DLT during the 8-week treatment, a further three subjects will be recruited at this dose level. Up to six subjects in total will then complete treatment at dose level C. If no further DLTs occur in this group of 6 subjects (i.e. 0-1/6 subjects with DLT), dose level C will be the MTD. If DLTs occur in two subjects in this group (i.e. 2/6 subjects with DLT), then dose level C has not been tolerated and the dose will be reduced to dose level B (see Section 9.3.2, page 23).

If two of the first three subjects treated at dose level C (2/3) experience a DLT during the 8-week treatment, then dose level C has not been tolerated and the dose will be reduced to dose level B (see Section 9.3.2, page 23).

The dose escalation schema is summarized in Table 2 (page 25).

9.3.4 Recruitment of Additional Subjects.

An additional 10 subjects will be treated at the MTD to further characterize the safety and efficacy of the study drug per the definition of MTD above.

Table 2: Rules for Dose Escalation and Allowable DLTs Per Cohort	
# DLT per cohort	**Dose escalation rule**
0/3	• Accrue the next 3 subjects at next dose level • If dose level C, accrue 3 additional subjects at this level
1/3	• Accrue 3 additional subjects at current dose level
2/3	• Current dose level has not been tolerated. Reduce dose to the lower dose level ○ If only 3 subjects have been treated at the lower dose level, accrue an additional 3 subjects at the lower dose level. ○ If 6 subjects have already been treated at the lower dose level, the lower dose level is the MTD. ○ If this occurs at dose level A, the study will be terminated early.
1/3 + 0/3 (1/6)	• Escalate dose. Accrue 3 new subjects at next highest dose level. If 0/6 or 1/6 DLTs at dose level C, then this is the MTD.
1/3 + 1/3 (2/6)	• Current dose level has not been tolerated. Reduce the dose. If this occurs at the lowest dose level, the study will be terminated early.
To be read with reference to Section 9.3 (page 22), above. Adapted from Koreth *et al*.[51]	

9.4 Determination of Maximum Tolerated Dose.

The maximum tolerated dose (MTD) is the highest tolerated dose level at which a minimum of 6 subjects have been evaluated, with fewer than 2 evaluable subjects in 6 experiencing a dose limiting toxicity (DLT); i.e. DLT in ≤1/6 evaluable subjects. **In addition to the above, at least 1 patient should meet the criteria for response or remission for it to be considered the MTD.**

Subjects will be considered unevaluable for DLT if they are removed from the study or die without developing DLT prior to receiving at least 8 weeks of IL-2 therapy. If there are fewer than 3 evaluable subjects in a cohort, additional subjects will be added at that dose level.

Intolerable lower grade toxicity that does not meet the definition of DLT, but still means that a subject is unable to complete the per-protocol treatment will be taken as equivalent to a DLT for the purpose of assessing dose escalation. If ≥2 subjects in a cohort of 3 refuse to continue at that dose level for at least 8 weeks due to intolerable lower grade toxicity, that dose level will not be regarded as the MTD.

9.5 Definition of Dose Limiting Toxicities.

Organ toxicities will be assessed at scheduled clinical visits and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0[54].

(i). **Anaphylaxis**: Life threatening anaphylaxis due to the study drug is considered a DLT.

(ii). **Grade 4 toxicity**: Grade 4 toxicity (except colitis, see below) directly related to IL-2 by week 8 of IL-2 treatment is considered a DLT.

(iii). **Grade 3 toxicity**: IL-2 will be withheld for grade 3 toxicity (except colitis).

(iv). **Development of a life-threatening infection**: Development of a life threatening infection, as assessed by the treating physician, prior to week 8 of IL-2 therapy will be considered a DLT. IL-2 will be withheld.

(v). **Grade 2 renal toxicity**: IL-2 will be withheld in the event of a grade 2 renal toxicity (creatinine = 1.5-3.0 x ULN).

Development of toxic megacolon or colitis requiring immediate surgical intervention is a well-described risk of active UC. Consequently, it will not be considered a DLT. However, IL-2 will be discontinued.

9.6 Clinical Assessments for Toxicity.

Subjects will undergo regular clinical examination (history and physical examination), blood tests and other tests as indicated at scheduled visits in order to detect potential drug toxicity, as detailed in Section 11 (Page 31).

9.7 Extended Treatment with Low-Dose IL-2.

After completing 8 weeks of IL-2 therapy, subjects who have achieved a clinical response or entered remission (see Section 14, Page 43, for definitions) at any dose level and have an acceptable toxicity profile will be allowed to continue treatment at that dose level, at the discretion of the treating physician. These subjects will be seen in clinic every 3 months for a year, after which they will be followed by the referring gastroenterologist. Prometheus Laboratories will continue to supply IL-2 for up to one year after completing 8 weeks of IL-2 therapy to subjects who have achieved a clinical response or entered remission at any dose level and have an acceptable toxicity profile.

Subjects on extended duration IL-2 therapy will not be evaluable for toxicity and DLT endpoints. The addition of other medications (except the combination of sirolimus and tacrolimus) will be permitted at the discretion of the treating physician. Subject assessment while on extended treatment with IL-2 is detailed in Section 11.4 (page 34). In the event of toxicity attributable to IL-2, therapy will be discontinued.

10 STUDY DRUG: ALDESLEUKIN.

10.1 Formulation, Reconstitution and Storage.

Recombinant human IL-2 (aldesleukin, Proleukin®) is supplied free of charge by Prometheus Laboratories, Inc. (San Diego, CA).

Aldesleukin is supplied as a sterile, white to off-white, lyophilized cake in single-use vials containing 22 million international units (MIU) of aldesleukin intended for intravenous (IV) administration. Vials of lyophilized aldesleukin will be stored in a refrigerator at 2-8°C (36-46°F) and will not be used beyond the expiration date printed on the label.

On reconstitution of a 22 MIU vial of aldesleukin with 1.2 ml of sterile water for injection (SWFI), each ml contains 18 MIU (1.1mg) aldesleukin, 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).

10.2 Reconstitution.

Each 22 MIU vial of aldesleukin should be reconstituted with 1.2 ml of sterile water for injection (SWFI). 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.

Reconstitution or dilution with Bacteriostatic Water for Injection or 0.9% Sodium Chloride for Injection should be avoided due to increased aggregates.

After reconstitution, the resulting solution should be a clear, colorless to slightly yellow liquid. Reconstitution and dilution procedures other than those described may alter the delivery and/or pharmacology of aldesleukin and are not recommended.

All syringes should be prepared in the pharmacy of the relevant institution at the same time, after diluting a 22 MIU aldesleukin vial with 1.2ml SWFI and 4.8ml D5W (final IL-2 concentration=3.6 MIU/mL), and any remaining product should be immediately discarded.

10.3 Storage.

Aldesleukin is an unpreserved sterile product. The reconstituted and diluted solutions should be stored in a refrigerator at 2-8°C (36-46°F). DO NOT FREEZE.

When reconstituted and diluted according to directions, aldesleukin is stable for up to 48 hours in plastic bags (e.g. PVC bags) when stored at refrigerated AND room temperatures (2-25°C [36-77°F]).

From a microbial point of view, the product should be used immediately. Vials should be entered only once for reconstitution to minimize the chances of contamination. If not used immediately, in-use storage times should normally not be longer than 24 hours at 2-8°C (36-46°F), unless reconstitution has been performed under controlled and validated aseptic conditions in a laminar airflow hood.

Data support the stability and sterility of reconstituted diluted aldesleukin preparations (reconstituted with SWFI and further diluted with D5W); and the stability and sterility of product reconstituted with SWFI but not further diluted, for up to 14 days at 2-8°C (36-46°F) when syringes are prepared by qualified health-care professionals under aseptic conditions[40]. Therefore, if reconstitution and dilution are performed under controlled and validated conditions using a laminar flow hood, the dose or doses thus prepared and stored at 2-8°C (36-46°F) need to be used within 14 days.

On 4/26/2016, the Investigational Drug Service (IDS) at Brigham & Women's Hospital, Boston MA, reduced the beyond use dating for aldesleukin that is prepared at their research pharmacy for this study from 14 days to 9 days. **Consequently, aldesleukin prepared at BWH IDS and subsequently stored at 2-8°C (36-46°F) needs to be used within 9 days.**

10.4 Dosage.

Aldesleukin will be given according to the dose schema described in Section 9.2 (page 21), based on the cohort into which they are accrued. The first two subjects to receive IL-2 will be admitted overnight to the Clinical Translational Study Unit (CTSU). Aldesleukin may be administered in the home or out-subject setting by daily subcutaneous injection for 8 weeks. Subjects who receive additional treatment after experiencing clinical benefit from 8 weeks of

initial therapy may continue aldesleukin therapy at their initial dose level. They will be followed by their physician according to Section 9.7 (page 27).

10.5 Administration.

The product should be inspected visually for particulate matter or discoloration and brought to room temperature before administration. Administer as a subcutaneous injection. DO NOT ADMINISTER AS INTRAVENOUS PUSH OR BOLUS. Premedications are not required prior to the first dose or escalated doses.

11 CLINICAL AND LABORATORY EVALUATIONS.

Participants will have 10 face-to-face encounters with research staff throughout the clinical trial, including (i) Screening at week -2, (ii) Baseline assessment prior to first dose (day 0), (iii) Clinical assessment prior to second dose (day 1), (iv-viii) Assessments at day 4 optional visit) and weeks 1, 2, 4 and 6, (ix) End of treatment assessment at week 8, and (x) End of study assessment at week 12. On completion of the study, research staff will contact participants by telephone monthly for 2 months and on a 2-monthly basis for a further two months. Assessments after week 1 (as indicated in Table 3, page 37) will be scheduled for the planned day ±3 days. Tests that would be performed as part of routine clinical care are highlighted as follows: "(routine)".

11.1 Screening (Pre-Treatment) Evaluation (Week -2).

Following signed, informed consent, the following screening evaluation will be performed within two weeks of the anticipated start date of aldesleukin therapy:

- Medical history including confirmation of fulfillment of inclusion criteria (routine).
- Physical examination, including vital signs, weight, height (routine).
- Pregnancy test for women of childbearing potential.
- Screening test for tuberculosis (TB): IFN-γ Release Assay (IGRA), e.g. QuantiFERON®. If the IGRA result is "indeterminate" or "positive", a CXR will be performed as part of routine clinical care to exclude pulmonary TB.
- Stool for culture and sensitivity (C&S), ova and parasites (O&P), *Clostridium difficile* stool toxin and fecal calprotectin (routine).
- Flexible sigmoidoscopy and biopsies with calculation of the Mayo score; or colonoscopy and biopsies if the subject has never had a full colonoscopy (routine). As part of routine clinical evaluation, two biopsies will be obtained when possible from each of the following sites: rectum, sigmoid colon, descending colon (n=up to 6). In addition, biopsies for research will be obtained from each site. From the descending colon, biopsies will be obtained to characterize lamina propria immune cells on fresh cells by flow cytometry

(n=2), transcriptomics (n=1), and for deeper immunologic and single cell analysis (n=5). From the sigmoid colon, biopsies will be obtained to characterize LPMCs on fresh cells by flow cytometry (n=2), transcriptomics (n=1), to cryomold for histologic assessment (n=2), and for deeper immunologic and single cell analysis (n=5). From the rectum, biopsies will be obtained to characterize LPMCs on fresh cells by flow cytometry (n=2), transcriptomics (n=1), mucosal microbiota (n=2), to cryomold for histologic assessment (n=2), and for deeper immunologic and single cell analysis (n=5).

- Hematology: Complete blood count (CBC) and differential (routine).
- Inflammatory markers: ESR and CRP (routine).
- Serum chemistries: glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, AST, ALT, and calcium (routine).
- Thyroid function tests (TSH, T3, free T4) (routine).
- Virology: serology for HIV, HBV and HCV.

Note: The following basic science investigations can be performed at the screening visit *or* at the baseline (Day 0) visit, at the discretion of the investigator.

- Immunology bloods: Quantitative flow cytometric assessment of leucocytes, NK cells and Treg subsets from PB and endoscopic biopsies. Assessment of PB and LP Treg function. Banking of PBMCs, plasma, RNA, DNA.
- Microbiology: Stool sample and mucosal biopsies for analysis of the intestinal microbiome.
- IL-2 serum levels and anti-aldesleukin antibodies.

11.2 Baseline, Treatment and End-of-Study Evaluations (Day 0 to Week 6 and Week 12).

The following evaluations will be performed at baseline (day 0), day 4 (optional visit), and weeks 1, 2, 4, 6 and 12:

- Medical history and physical examination, including assessment of symptoms and signs in order to detect potential drug toxicity (routine).

- Hematology: Complete blood count (CBC) and differential (routine).

- Inflammatory markers: ESR and CRP (routine).

- Serum chemistries: glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, AST, ALT, and calcium (routine).

- Thyroid function tests (TSH, T3, free T4) (week 12).

- Immunology bloods: Quantitative flow cytometric assessment of leucocytes, NK cells and Treg subsets from PB. Assessment of PB and LP Treg function. Banking of PBMCs, plasma, RNA, DNA.

- Microbiology: Stool sample for analysis of the intestinal microbiome (day 0, weeks 1, 2, 4, 6, 8 and 12).

- Fecal calprotectin (day 0, weeks 1, 2, 4, 6, 8 and 12).

- IL-2 serum levels and anti-aldesleukin antibodies (not days 1 or 4).

Participants will attend the CTSU for their first IL-2 dose on day 0 and their second IL-2 injection on day 1. Participants will be evaluated clinically at that encounter.

11.3 End of Treatment Evaluation (Week 8).

The following evaluations will be performed at week 8:

- Medical history and physical examination, including assessment of symptoms and signs in order to detect potential drug toxicity (routine).

- Flexible sigmoidoscopy and biopsies with calculation of the Mayo score. The same biopsies will be obtained as at the screening procedure, except that histology will be limited to the rectum and sigmoid/distal descending colon.

- Hematology: Complete blood count (CBC) and differential (routine).

- Inflammatory markers: ESR and CRP (routine).
- Serum chemistries: glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, AST, ALT, and calcium (routine).
- Thyroid function tests (TSH, T3, free T4).
- Immunology bloods: Quantitative flow cytometric assessment of leucocytes, NK cells and Treg subsets from PB. Assessment of PB and LP Treg function. Banking of PBMCs, plasma, RNA, DNA.
- Microbiology: Stool sample and mucosal biopsies for analysis of the intestinal microbiome.
- IL-2 serum levels and anti-aldesleukin antibodies.

11.4 Evaluation of Subjects Undergoing Extended Treatment with Low Dose IL-2.

Patients with a treatment response who elect to undergo extended treatment (as described in Section 9.7, page 27) will be seen in clinic every 3 months as part of their routine clinical care. Evaluation may include:

- Medical history and physical examination, including assessment of symptoms and signs in order to detect potential drug toxicity (routine).
- Hematology: Complete blood count (CBC) and differential (routine).
- Inflammatory markers: ESR and CRP (routine).
- Serum chemistries: glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, AST, ALT, and calcium (routine).
- Immunology bloods: Quantitative flow cytometric assessment of leucocytes, NK cells and Treg subsets from PB. Assessment of PB and LP Treg function. Banking of PBMCs, plasma, RNA, DNA.

11.5 Details of Specific Assays.

Routine laboratory tests will be performed at Boston Children's Hospital, Labcorp or other facilities according to availability.

The techniques required for immune phenotyping (e.g. FACS, PCR etc.) will be performed on appropriately collected samples at the Snapper Laboratory at Boston Children's Hospital, the Harvard Digestive Disease Center and collaborating laboratories. The laboratory personnel who will perform these assays will be identified and/or appointed prior to the inception of this study. The specific protocols for each investigation, including the number of cells needed for each assay and precise locations and numbers of endoscopic biopsies etc., will be placed in the Trial Master File. A maximum of 26 biopsies will be obtained from each participant at each endoscopy for the purpose of these investigations, as described in Section 11.1 (page 31).

- Quantification of leucocytes and Treg subsets will be performed by flow cytometric identification of CD3, CD4, CD8, CD25, CD127, CD45RA and FOXP3 in PBMC and LPMC preparations. CD16 and CD56 expression will be used to identify NK cells.

- The biological effect of SC IL-2 on STAT5 phosphorylation in effector T cells and Tregs will be assessed by flow cytometry.

- The suppressive function of PB and LP Tregs prior to and during treatment will be evaluated by assessment of Treg-mediated inhibition of CFSE dilution, using sorted $CD4^{+}CD25^{hi}CD127^{lo}$ Tregs and freeze-thawed CFSE-labelled allogeneic $CD4^{+}CD25^{-}$ T responders.

- Intestinal microbiome analysis will be performed on snap-frozen stool and endoscopic biopsy samples. This will include phylogenetic identification and subtyping based on 16S ribosomal RNA classification and will be performed at the Harvard Digestive Disease Center Microbiome Core Facility.

11.6 Assessment of Aldesleukin Immunogenicity.

IL-2 levels and anti-aldesleukin antibodies will be determined at baseline, weeks 1, 2, 4, 6, 8 and 12. If anti-aldesleukin antibodies are detected, anti-aldesleukin antibody levels will be monitored beyond the 12-week study period until antibody levels have returned to baseline.

Table 3: Summary of Required Data				
	Screening (Pre-treatment)	Days 0, 1[a], 4[b] Weeks 1, 2, 4, 6	Week 8	Week 12
Medical history				
Physical examination				
Pregnancy test				
Viral testing: HIV, HBV, HCV				
Screening for TB				
Stool for C&S, O&P, *C. difficile*				
Colonoscopy/flexible sigmoidoscopy[1] & Mayo score				
CBC & differential		*		
ESR, CRP		*		
Serum chemistry		*		
Thyroid function tests				
IL-2 serum levels		*		
Anti-aldesleukin antibodies		[2]		
Intestinal microbiome analysis[4]		[2]		
Fecal calprotectin		[3]		
Immune phenotyping		*		

Notes. a: History and physical examination only at day 1, unless additional investigations indicated clinically. b: Day 4 visit is optional.

1: A full colonoscopy may be performed at screening to define disease extent in addition to mucosal inflammation if the subject has never had a full colonoscopy.

2: Not days 1 or 4. 3. Not day 1.

11.7 Anticipated Toxicities.

The toxicities seen in clinical trials utilizing similar doses of IL-2 are shown in Table 4, below. They are also discussed in Section 4.6 (page 13).

Table 4: Anticipated Toxicities

Study	IL-2 dose	Indication	Evaluable subjects (n)	Observed toxicities	n	(%)
Soiffer *et al.* [50]	0.2-0.6x10^6 IU/m^2/day IV	Enhance graft *vs.* leukemia effect following HSCT	29	Fatigue	15	(52)
				Temp >38°C	14	(48)
				Nausea/vomiting	11	(38)
				Myalgia	9	(31)
				Rash	9	(31)
				Diarrhea	7	(24)
				Edema	6	(21)
				Dyspnea	6	(21)
				Catheter infection	5	(17)
				Thyroid dysfunction	2	(7)
				Thrombocytopenia	2	(7)
Koreth *el al.* [51]	0.3-3x10^6 IU/m^2/day SC	Treatment of steroid-resistant GvHD	28	Injection site induration	3	(11)
				Infections*	3	(11)
				Renal failure**	2	(7)
				Renal dysfunction	1	(4)
				Flu-like symptoms	1	(4)
				Thrombocytopenia	1	(4)
Saadoun *et al.* [52]	1.5-3x10^6 IU/day SC	Treatment of HCV-associated vasculitis	10	Fatigue	4	(40)
				Flu-like symptoms	4	(40)
				Injection site induration	2	(20)
				Arterial hypertension	1	(10)
				Myalgia	1	(10)

* Recurrent MRSA pneumonia (1), *Hemophilus influenzae* type-B bacteremia (1), MRSA furuncle (1)

** Thrombotic microangiopathic renal failure in two subjects taking tacrolimus and sirolimus. This is a recognized complication of this combination of medications.

11.8 Criteria for Treatment Discontinuation.

Subjects will be removed from the study and therapy with IL-2 should be abandoned for any of the following circumstances if they are deemed possibly, probably or definitely related to IL-2, or per subject preference:

- The subject withdraws his/her consent at any time.
- Anaphylactic reaction to IL-2.
- Life threatening infection on IL-2.
- Other grade 4 toxic event.
- Grade 3 toxicity.
- Grade 2 renal toxicity.
- The development of toxic megacolon or colitis requiring immediate surgical intervention.
- At the discretion of the treating physician.

11.9 Dose Modification/Toxicity Management.

In the event of IL-2 related toxicity that does not meet the requirement for treatment discontinuation (grade 2 toxicity or less, excluding grade 2 renal toxicity), the treating physician may elect to withhold IL-2 to allow the toxicity to resolve. IL-2 can be recommenced at 50% of the target dose if the toxicity resolves to grade 1 or better within 2 weeks. At the discretion of the treating physician, if re-introduction of IL-2 is tolerated by the subject, the dose of can be escalated to the target dose.

12 REPORTING OF UNANTICIPATED PROBLEMS INVOLVING RISKS TO RESEARCH SUBJECTS, INCLUDING ADVERSE EVENTS.

12.1 Reporting to the Principal Investigator.

The following events will be reported to the principal investigator within 24 hours of being known:

- Death of a research subject.
- Dose-limiting toxicity.
- Adverse event that is unexpected and related or possibly related to the subject's participation in research.
- Medication or laboratory errors.
- Breach of confidentiality or HIPAA violation.
- Significant protocol deviation or non-compliance.
- A research-related complaint.
- Intentional change to protocol without IRB approval, to eliminate apparent immediate hazard to research subject.
- Enforcement action.
- Study personnel misconduct.
- Incarceration of a research subject during study participation.
- Any other event that the investigator thinks may represent an unanticipated problem that involves risks to subjects or others.

12.2 Reporting to the Institutional Review Board.

Consistent with institutional guidance, the principal investigator will use a CHeRP "Unanticipated Problem or Event Form" to report the following events to Boston Children's Hospital's Committee on Clinical Investigation within 72 hours of being known:

- Death of a research subject, thought to be related to the research study or possibly related to the research study.
- Adverse event that is both unexpected and related or possibly related to the subject's participation in research.
- Medication or laboratory errors.
- Breach of confidentiality or HIPAA violation.
- Significant protocol deviation or non-compliance.
- A research-related complaint.
- Intentional change to protocol without IRB approval, to eliminate apparent immediate hazard to research subject.
- Interim findings, publication or safety report.
- Enforcement action.
- Study personnel misconduct.
- Incarceration of a research subject during study participation, so that additional IRB review can be accomplished in order for the participant to remain in the study.
- Any other event that the investigator thinks may represent an unanticipated problem that involves risks to subjects or others.

12.3 Reporting to Prometheus Laboratories.

Prometheus Laboratories will supply the study drug free-of-charge. The principal investigator will transmit an anonymized copy of any serious adverse event reports (or FDA Form 3500A MedWatch reports, if applicable) to Prometheus Laboratories.

Prometheus Drug Safety Email: drugsafety@prometheuslabs.com

Prometheus Drug Safety Fax: (858) 754-3046

13 DATA SAFETY AND MONITORING BOARD.

A Data Safety and Monitoring Board (DSMB) has been constituted. The DSMB members are as follows: Hans Herfarth MD PhD (Chair, Gastroenterologist, University of North Carolina at Chapel Hill), Ashwin Ananthakrishnan MB BS MPH (Gastroenterologist, Massachusetts General Hospital), and Tanya Logvinenko PhD (Statistician, Boston Children's Hospital). The DSMB will determine stopping points for individual subjects and the study as a whole. In addition, the DSMB will determine the schedule of meetings in order to review subject safety information and will also make recommendations concerning the continuation, modification or termination of the study.

The DSMB will review study data and make recommendations on the conduct of the study at the following time points:

- In the event of any serious adverse events or DLT.
- When the third subject completes 8 weeks of IL-2 treatment, as per protocol.
- Three months after study initiation.
- Six months after study initiation.
- At six month intervals thereafter.

14 STUDY ENDPOINTS.

Subjects will be periodically assessed as described in Section 11 (page 31) in order to determine if the primary and secondary endpoints have been met.

14.1 Determination of Safety of Daily SC Low Dose IL-2.

The safety of daily low-dose IL-2 will be determined by assessing for potential side-effects and dose limiting toxicity at each study visit.

14.2 Determination of the Maximum Tolerated Dose of Daily SC Low Dose IL-2.

The MTD will be determined using the dose escalation strategy and assessments described in Sections 9 and 11 (pages 21 and 31, respectively).

14.3 Determination of the Feasibility of Administering an Eight Week Course of Daily SC Low Dose IL-2 in Subjects with Moderate to Severe UC.

This relates to the technical feasibility of administering daily low-dose SC IL-2 as per protocol. The narrative report of this outcome will include the numbers of subjects enrolled, subjects who completed the protocol and a description of any barriers to the technical feasibility of this approach in subjects with UC.

14.4 Determination of the Clinical Response to Daily SC Low Dose IL-2.

Mayo score[7,55] (Table 5; page 44) will be assessed as per protocol between weeks -2 and 0 (as close to week 0 as possible) and week 8 in order to determine clinical response. Moderate to severe UC, denoted by a Mayo score of 6-12 is an inclusion criterion. Clinical response and remission are defined according to Rutgeerts *et al.*[7]

14.4.1 Definition of Clinical Response.

> *"A decrease from baseline in the total Mayo score of at least 3 points and at least 30% , with an accompanying decrease in the subscore for rectal bleeding of at least 1 point or an absolute subscore for rectal bleeding of 0 or 1."* [7]

14.4.2 Definition of Remission..

"A total Mayo score of 2 points or lower, with no individual subscore exceeding 1 point."[7]

14.4.3 Definition of Mucosal Healing.

"An absolute subscore for endoscopy of 0 or 1."[7]

14.5 Assessment of the Immunological Response to IL-2 Therapy.

Data obtained from research laboratory-based assessment of subject immune responses to IL-2 therapy, that do not have direct clinical relevance, will be reported as appropriate for each data type.

Table 5: Mayo Scoring System for Assessment of Ulcerative Colitis Activity. [7,55]

Stool frequency*	
0	Normal no. of stools for this subject
1	1 to 2 stools more than normal
2	3 to 4 stools more than normal
3	5 or more stools more than normal
Rectal bleeding**	
0	No blood seen
1	Streaks of blood with stool less than half the time
2	Obvious blood with stool most of the time
3	Blood alone passes
Findings on endoscopy	
0	Normal or inactive disease
1	Mild disease (erythema, decreased vascular pattern, mild friability)
2	Moderate disease (marked erythema, lack of vascular pattern, friability, erosions)
3	Severe disease (spontaneous bleeding, ulceration)
Physician's global assessment	
0	Normal
1	Mild disease
2	Moderate disease

3	Severe disease

* Each subject serves as their own control.
** Represents the most severe bleeding of the day.

15 STATISTICAL CONSIDERATIONS.

The primary endpoint of this Phase I study is to determine the MTD of daily subcutaneous IL-2 in moderate to severe UC.

15.1 Probability of Dose Escalation Under Various DLT Rates.

Table 6 shows the probability of escalation under various true DLT rates. With this design, there is 91% probability of escalation if the true rate of DLT is 10% and 17% probability of escalation if the true DLT rate is 50%.

Table 6: Probability of Dose Escalation

True rate of DLT	Probability of dose escalation
10%	0.91
20%	0.71
30%	0.49
40%	0.31
50%	0.17
60%	0.08

Once the MTD is established, an additional 10 evaluable subjects will be treated at the MTD level to further evaluate the toxicity as well as the feasibility. The estimation of toxicity rate will be based on these 10 additional subjects. With 10 subjects, the 90% confidence interval of the toxicity rate will be within ±26%.

15.2 Accrual.

We anticipate that the sample size will range from 3-28 evaluable subjects, depending on number of dose levels will be tested. Based on the current practice, we anticipate that the accrual rate will be approximately 12 subjects per year, thus requiring up to 28 months to complete accrual.

16 ETHICS AND GOOD CLINICAL PRACTICE.

This study will be performed in accordance with Boston Children's Hospital's policies and guidance on the conduct of clinical research, the principles of Good Clinical Practice (GCP), the Declaration of Helsinki and applicable state and federal laws and regulations.

The study may not start without written Institutional Review Board approval and the written informed consent of the subject.

17 REFERENCES.

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18 APPENDIX A: MAYO SCORING WORKSHEET.

Circle only one of each subscore and enter in equation below. The worst diary entry from the three days prior to each study visit will be used for each subject-reported subscore.

1. **Stool Frequency Subscore***

Normal number of bowel movements per day when UC is NOT active (transcribed from medical history): _____

0 – Normal number of stools for this subject

1 – One to two stools more than normal

2 – Three to four stools more than normal

3 – Five or more stools more than normal

* Each subject serves as his or her own control to establish normal stool frequency and the degree of abnormal stool frequency.

2. **Rectal Bleeding Subscore ****

0 – No blood seen

1 – Streaks of blood with stool less than half the time

2 – Obvious blood with stool most of the time

3 – Blood alone passed

** The daily bleeding score represents the most severe bleeding of the day.

3. **Physician's Global Assessment Subscore *****

0 – Normal (subscores are 0)

1 – Mild disease (subscores are mostly 1's)

2 – Moderate disease (subscores are mostly 1 to 2)

3 – Severe disease (subscores are mostly 2 to 3)

*** The physician's global assessment acknowledges the three other subscores, the subject's daily record of abdominal discomfort and functional assessment, and other observations such as physical findings, and the subject's performance status.

4. Endoscopy Subscore: Findings of colonoscopy/flexible sigmoidoscopy

0 – Normal or inactive disease

1 – Mild disease (erythema, decreased vascular pattern, mild friability)

2 – Moderate disease (marked erythema, absent vascular pattern, friability, erosions)

3 – Severe disease (spontaneous bleeding, ulceration)

Complete applicable score for the visit.

Partial MAYO Score

_____	+	_____	+	_____	=	_____
Subscore 1		Subscore 2		Subscore 3		Partial MAYO Score

MAYO Score

_____	+	_____	=	_____
Partial MAYO Score		Subscore 4		Mayo Score

19 APPENDIX B: COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS.

The National Cancer Institute's "Common Terminology Criteria for Adverse Events" (CTCAE) v4.03 is attached for reference. CTCAE toxicities are graded on a scale of 0 to 4. If a specific event is not included in the toxicity scale, the following scale should be used to grade the event:

Grade	Definition
0	**No toxicity**
1	**Mild toxicity** (usually transient, requiring no special treatment and generally not interfering with usual daily activities, e.g., colds)
2	**Moderate toxicity** (usually ameliorated by simple therapeutic manoeuvres, and impairs usual activities, e.g., pneumonia treated as an out-patient)
3	**Severe toxicity** (requires vigorous therapeutic intervention, interrupts usual activities. Hospitalization may or may not be required, e.g., pneumonia requiring hospitalization or intravenous antibiotics)
4	**Life-threatening toxicity** (requires hospitalization, e.g., immediate risk of death from event and not reactions that had it occurred in a more serious form, might have caused death)