AIDS 400

HIV Reservoir Reduction with Interleukin-2

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SYNOPSIS

Introduction

Interleukin-2 (IL-2) has been extensively studied in HIV infected individuals with no demonstrated clinical benefit as far as improving survival or decreasing risk of progression to AIDS. The results of the two landmark, international, multicenter, phase III, randomized trials of IL-2 in HIV infected participants (SILCAAT and ESPRIT) have been recently published. These trials, which started more than a decade ago, enrolled over 5800 participants who were randomized to anti-retroviral therapy (ART) +/- IL-2 and showed no benefit to IL-2 treatment in survival or progression to AIDS. Many individuals with HIV infection can lead normal lives on ART but replication-competent virus remains within resting CD4+ cells, referred to as the "HIV reservoir". There is a renewed interest in strategies to decrease or eliminate the viral reservoir in an attempt to provide a sterilizing or a functional "cure" for HIV that would allow the discontinuation of ART, which currently must be taken life-long. These therapies have long-term metabolic and cardiovascular toxicities as well as substantial cost. More recent data suggest that IL-2 administration may decrease the size of the HIV reservoir, getting ART-treated participants closer to levels of HIV persistence that may ultimately allow for sterilizing or functional cure.

Purpose

The purpose of this pilot study is to examine the effects of eight 4-day cycles of subcutaneous recombinant interleukin-2 (rIL-2) given every 8 weeks on levels of replication-competent HIV in CD4 cells and on the size of HIV viral reservoir in up to 20 participants with chronically suppressed HIV infection (viral load <50 copies/mL).

Design

Pilot open label trial of rIL-2 administration.

Power and Sample Size Considerations

We base our sample size calculations on the primary analysis using a mixed effect model to estimate the effect of rIL-2 administration on the magnitude of the inducible reservoir by QVOA. In the Chun experiment (see below), the difference between the IL-2 and the HAART-only group was 0.64 IUPM, and the variance in the IL-2 group was 0.41. In a 200-simulation run, we estimate that a total sample size of 16 participants will provide an 80% power (95% CI, 0.74-0.84) to detect a difference from baseline to post-IL-2 administration as large as that seen in the Chun paper. Of note, these estimates are likely conservative, since the variation observed in repeated measurements with the optimized version of the QVOA assay that will be used in this study may be lower (variance around 0.11) than that of the assay used in the original Chun report (1).

1 Accelevir, data on file

Inclusion Criteria

Participants may enroll in the study if they meet the following criteria:

1. Written informed consent signed and dated by study participant.
2. Male or female, at least 18 years of age and not older than 65 years of age.
3. HIV-1 infection, documented by and FDA-approved ELISA, EIA, or rapid antibody detection method, and confirmed by a second approved antibody-based test or by a positive approved HIV RNA detection assay.
4. CD4+ T cell count ≥ 350 cells/mm^3.
5. HIV-1 RNA < 50 copies/mL obtained within 60 days prior to study entry performed with an FDA-approved HIV-1 RNA assay.
6. Adequate venous access and no other contraindications for leukapheresis.
7. Absolute neutrophil count (ANC) $\geq$ 1500/mm^3.
8. Hemoglobin level >10 g/dL.
9. Platelet count $\geq$100,000/mm^3.
10. Serum creatinine $\leq$1.5 mg/dL
11. AST and ALT <2.5 times the upper limit of normal
12. TSH, T3, and T4 levels within the normal range of the processing laboratory.
13. Anti-thyrosine peroxidase (TPO) within the normal range of the processing laboratory.
14. Willing to comply with study-mandated evaluations; including not changing antiretroviral regimen (unless medically indicated) during the study period.
15. All participants must have received HAART, and had viral loads below the limit of quantification of the assay for at least 1 year. Participants who had intermittent isolated episodes of detectable low-level viremia < 500 copies RNA/mL flanked by viral loads below the limit of quantification of the assay will remain eligible.
16. On a stable combination antiretroviral regimen (no changes to treatment within 4 weeks of enrollment) including at least 3 medications and willing to continue on current antiretroviral therapy for the duration of the study, unless otherwise medically indicated.

Exclusion Criteria

1. Childbearing potential for female participants. For the purposes of this study, a woman is considered to be of childbearing potential if she is postmenarchial, has not had a documented surgical sterilization procedure, has an intact uterus and at least 1 ovary, and has had a spontaneous menstrual period in the last 2 years.
2. Acute or chronic hepatitis C infection, defined as a positive plasma HCV RNA using any FDA-approved qualitative or quantitative test in a participant with a positive HCV antibody (HCV RNA testing is not required in participants with a negative HCV antibody). Participants who have completed a course of a direct-acting antiviral agent for hepatitis C and have a confirmed plasma HCV RNA level below the limit of detection of the assay 12 weeks or longer after completion of therapy will be eligible.
3. Acute or chronic hepatitis B infection, defined as a positive HBV surface antigen or a positive HBV DNA.
4. History of advanced chronic liver disease, including cirrhosis, advanced liver fibrosis, severe portal hypertension, or manifestations or hepatic failure.
5. History of malignant disease that is not considered to be surgically or medically eradicated or that has required any form of therapy in the past 5 years.

6. Current diagnosis of congestive heart failure of any severity, uncontrolled angina or uncontrolled arrhythmias.
7. History of chronic lung disease that has required pharmacologic treatment, oxygen supplementation, medical monitoring, or hospitalization in the previous year, or that is expected to cause persistent or recurrent pulmonary symptoms or impairment. Examples of the latter include but are not limited to chronic bronchitis, emphysema, and pulmonary fibrosis.
8. History or any features on physical examination indicative of a bleeding diathesis.
9. History or current diagnosis of thromboembolic disease, including deep vein thrombosis and pulmonary embolism, or family history of the same.
10. History of hypersensitivity to radiological contrast media or anticipated need for exposure to radiological contrast media during the study period.
11. Use of chronic corticosteroids, hydroxyurea, or immune-modulating agents (e.g., interleukin-2, interferon-alpha or gamma, granulocyte colony stimulating factors, etc.) within 30 days prior to enrollment.
    NOTE: Use of inhaled or topical steroids is not exclusionary.
12. Breast-feeding.
13. Use of aspirin, warfarin or any other antithrombotic or antiplatelet agent during the 2-week period prior to leukapheresis.
14. History of autoimmune disorders, including but not limited to Crohn's disease, scleroderma, thyroiditis, inflammatory arthritis, myasthenia gravis, glomerulonephritis, systemic lupus erythematous, and vasculitis.
15. Type 1 diabetes mellitus.
16. History of thyroid disease that has required antithyroid or thyroid hormone replacement therapy at any time in the past.
17. Current continued use, or anticipated continued medical indication for nephrotoxic agents, including but not limited to aminoglycosides and other potentially nephrotoxic antimicrobials, indomethacin, high scheduled doses of other NSAIDs, and lithium salts.
    NOTE: Low doses or limited duration of these agents (i.e., ≤14 days) is not exclusionary.
18. Current continued use, or anticipated continued medical indication for hepatotoxic agents, including but not limited to amiodarone, methotrexate, and anticonvulsants and antimicrobials with elevated hepatotoxic potential.
    NOTE: Low doses or limited duration of these agents (i.e., ≤14 days) is not exclusionary.
19. Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.
20. Serious illness requiring systemic treatment and/or hospitalization within 30 days prior to study entry that in the judgement of the investigator may compromise study participation or pose additional risks to the participant.
21. Any other condition that, in the opinion of the clinical investigator or sponsor, might compromise any aspect of this trial.

Study Treatment

All 20 participants will receive subcutaneous rIL-2 therapy in addition to combination antiretroviral therapy. Any combination of three FDA-approved antriretrovirals may be used.
Recombinant IL-2 at a dose of 5 MIU twice daily will be given subcutaneously for 4 consecutive days every 8 weeks for eight cycles.

Blood will be drawn for CD4 cell counts, for viral load, for routine safety monitoring and for HIV reservoir assays as detailed in section D.3.5 of the protocol.

Safety Monitoring

rIL-2 has been given safely to HIV infected persons. Study participants will be monitored by phone contact for safety and tolerance by study staff on days 2 and 4 of each IL-2 cycle. Before each rIL-2 cycle and on day 7 of several of the IL-2 cycles and whenever else indicated clinically, safety laboratories will be obtained on all study participants. Nursing staff and physicians are available 24 hours per day, 7 days per week to deal with emergent toxicities.

Study endpoints and outcome measures

The primary endpoint of the study is the change in the level of replication-competent virus from baseline to the end of rIL-2 treatment, as measured by the Quantitative Viral Outgrowth Assay (QVOA).

Primary Objective

The primary objective will be to compare levels of replication competent HIV in circulating CD4 T cells before and after up to 8 cycles of IL-2 administration. The frequency of CD4 T cells containing replication-competent virus in leukapheresis samples obtained at baseline and at the end of IL-2 treatment will be enumerated using several state of the art methods. The primary readout for this objective will be measurement of replication-competent virus by Quantitative Viral Outgrowth Assay (QVOA) performed by Robert Siliciano's group at Accelevir.

Secondary Objectives

1) To examine the relationships between levels of replication competent HIV as identified by QVOA and other measures of HIV persistence:
   a) the intact proviral DNA assay (Siliciano),
   b) the Envelope Detection by Induced Transcription-based Sequencing (EDITS, Karn) performed on the same pre-trial and post-trial leukapheresis samples.
2) To explore the effects of IL-2 administration on induction of HIV expression in vivo as measured by the EDITS assay and plasma HIV levels.
3) To examine the effects of IL-2 administration on natural killer cell phenotype and function and on the ability of NK cells to limit HIV propagation in vitro.

A. INTRODUCTION

A.1. BACKGROUND

Highly active anti-retroviral therapy (HAART) has been successful in controlling viral replication and decreasing levels of virus in plasma to levels below limits of detection as determined by commercially available assays in most human immunodeficiency virus (HIV)-infected individuals (1-3). Even in individuals who have been treated for considerable periods of time and whose plasma virus levels remain below detectable, replication-competent HIV can be invariably isolated from latently infected, resting CD4+ T cells (4-6). This observation, and the common experience of viral rebound among HIV-infected individuals in whom HAART has been discontinued (7), as well as the fact that this pool of latently infected cells is long-lived and does not decay substantially over time (4, 5), have led to the recognition that these cells constitute a principal impediment to the "cure" of HIV infection (4-6, 8-10).

A variety of cytokines, including interleukin (IL)-2, tumor necrosis factor (TNF)-α IL-15 and IL-6 can induce in vitro the expression of HIV from latently infected, resting CD4+ T cells obtained from HIV infected individuals (11). Thus, it has been proposed that latent HIV might be deliberately 'purged' by treating infected individuals simultaneously with HAART and agents that activate cells to express HIV (8-10, 12). After being activated, these latently infected cells may die from cytopathic effects and/or immune effector mechanisms, whereas HAART would prevent new rounds of infection mediated by the replication competent viruses expressed by activation from latency (8-10, 12).

Many novel strategies are being explored in an effort to completely eradicate HIV infection. The majority will likely fail and, for the reasons outlined above, even those that reliably decrease the size of the HIV reservoir may be insufficient to cure HIV. We still do not have a sense that there exists a level of reservoir reduction — without complete elimination — that would suffice to result in durable control of HIV as defined by the sustained absence of viremia after discontinuation of antiretroviral therapy. In this regard, the limits of our ability to monitor virus persistence make it impossible to know if every infectious virus has been eliminated from the one person who has been cured. In that patient, replacement of the entire immune cell pool with cells resistant to HIV likely rendered the emergence from latency of any residual infectious virus incapable of amplification. There is reason to suspect that administration of interleukin-2 may have significant impact on the size of the HIV reservoir.

A.2. INTERLEUKIN-2 AND THE HIV RESERVOIR

Exogenous administration of interleukin-2 has been explored as a strategy to purge HIV from latently infected CD4+ T cells and thus reduce the viral reservoir (13-15). The rationale for this approach was the observation that in a pooled analysis of three of the phase II randomized studies of rIL-2 (16) the mean decrease from baseline plasma HIV RNA was significantly greater in patients randomized to receive rIL-2 plus ART versus ART alone, and that in vitro IL-2 can be used to activate latent integrated virus (13). Chun et al reported a cross sectional analysis of reservoir size among 26 ART treated participants who had been treated with ART alone or ART plus an average of 10 cycles of interleukin-2 (IL-2). IL-2 had been administered with an eye to increasing circulating CD4+ T cells as was the

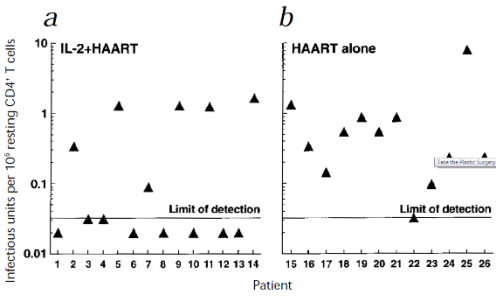

Fig. 1 Frequency of resting CD4⁺ T cells containing replication-competent HIV-1. Patients receiving IL-2 plus HAART (***a***) or HAART alone (***b***) were assessed by activation of purified resting CD4⁺ T cells on day 0 (with duplicates of 10 million cells per well) and determination of IUPM (vertical axis). Horizontal axis, patient identification number.

rationale for this therapy at that time. Fourteen of these participants received ART plus IL-2 and 12 received ART alone. At the time of "reservoir" analysis all participants were receiving combination ART and had plasma HIV levels <50 copies/mL. Shown in Figure 1 from this publication (13), eight of 14 ART plus IL-2-treated participants had a frequency of CD4 T cells in peripheral blood that contained replication-competent HIV (as detected by cocultivation assay) at or below the lower limit of detection ($0.03/10^6$ IUPM) while only one of 12 participants who had received ART alone had replication competent virus levels that low. Two participants in the ART plus IL-2 group also had an examination of lymph node CD4+ T cells and monocyte/macrophages (4-5 x 10^7 cells) and virus could not be found by cocultivation assay. Encouraged by these findings, Chun et al discontinued antiviral therapy in these two participants. Despite undetectable virus levels in plasma and the inability to find replication competent virus in blood and lymph nodes, viremia recurred in both, indicating that cure was not achieved (17) and providing early indication, more recently confirmed by other groups (18) that our assays for detection of replication competent virus in blood and tissue are not now (nor likely ever to be) sufficiently sensitive to exclude levels of virus in the body that are capable of rebound. Nonetheless, an effect on reservoir size as detected by these assays is a useful finding from which to proceed in a step-wise fashion to the ultimate objective of complete reservoir eradication. A demonstrable effect on this reservoir is a valuable addition to ultimate goal of HIV eradication. As these published studies have never been replicated, our goal is to confirm these important observations in a prospective study specifically designed to evaluate the effects of IL-2 infusion on indices of HIV persistence. As noted above, interleukin-2 has been studied intensively for its ability to increase circulating CD4 T cells in vivo and was examined in two large clinical trials (ESPRIT and SILCAAT) to see if this CD4 T cell increase conferred clinical benefit. These controlled studies, enrolling nearly 6,000 patients, could show no clinical benefit of IL-2 administration (19). We are proposing to examine the effects of IL-2 administration to ascertain if this strategy can decrease the size of the reservoir of replication competent HIV. To our knowledge, there have been no studies attempting to reproduce the Chun work and we propose that a carefully designed trial is an important priority for HIV eradication research because the effect on the HIV reservoir as reported by Chun is substantial and because cycles of IL-2 infusion have manageable toxicity, have been given safely to thousands of HIV infected persons, can be administered in an outpatient setting, and thus are readily scalable.

There is growing recognition that natural killer (NK) cells play an important role in the course of HIV disease as genetic studies have linked polymorphisms in surface receptors expressed by NK cells - killer cell immunoglobulin-like receptors (Kirs) and HLA antigens to which they can bind - to differential outcomes of HIV disease in natural history studies (20, 21). In early studies, we demonstrated significant impairment in NK cell function in HIV infected persons (22) and later, we and others have shown that this could be improved *in vitro* by exposure to interleukin-2 (23, 24). More to the point, clinical trials of IL-2 administration *in vivo* have found that IL-2 administration increases NK cell activity [but decreases the cytolytic activity of HIV-specific CD8 T cells (25)]. Thus, we propose that *in vivo* administration of IL-2 will both activate HIV expression from latency among memory CD4 T cells and concurrently will activate NK cells. NK cells are recognized to limit HIV replication in vitro and this may be mediated by direct cytolytic activity as well as by the elaboration of type 1 and type 2 interferons and beta chemokines (CCL3, CCL4, CCL5) that by different mechanisms can block HIV spread in tissues.

The hypothesis being tested in this pilot trial is that intervention with intermittent IL-2 therapy in combination with antiretroviral therapy among patients with controlled HIV infection on a stable HAART regimen will reduce the reservoir of replication competent HIV in blood.

A.3. SUBCUTANEOUS rIL-2

Interleukin-2 (IL-2) is a T-cell growth factor and is integral to the function of the immune system. Recombinant interleukin-2 (rIL-2), with the trade name Proleukin®, is a potent immunomodulator currently licensed for the treatment of metastatic renal cell carcinoma and metastatic melanoma. Originally described as T-cell growth factor, this natural protein exerts potent in vitro effects on cells derived from patients with HIV. Critically, IL-2 induces proliferation of T-lymphocytes and B-lymphocytes, enhances cytolytic activity against a variety of target cells, increases production of gamma-interferon, increases secretion of gamma-globulins and importantly for this trial, increases natural killer cell function (26). Initial clinical studies coupled with laboratory evaluations identified a regimen for optimal induction of CD4+ T-lymphocyte proliferation (27). Intermittent administration of rIL-2 to patients with HIV infection based upon a regimen delivered for five days every 8 weeks has now been examined in multiple trials (as detailed below).

A.4. CLINICAL EXPERIENCE WITH SUBCUTANEOUS rIL-2 IN HIV INFECTION

Phase I studies of exogenous IL-2 in HIV infection were initiated as early as 1983, before the availability of HAART. These initial trials were conducted with the native protein purified from T-cell cultures. They have demonstrated that the intermittent administration of rIL-2 either as a continuous intravenous infusion or subcutaneously (usually given twice daily for 5 consecutive days) with HAART result in substantial and significantly higher CD4+ T-cell increases than those achieved with either dual nucleoside reverse transcriptase inhibitor therapy or more potent HAART alone (28). To summarize:

- rIL-2 plus ART produces significant CD4+ T-cell expansion compared with ART alone; it is noteworthy that many of these phase II studies preceded the use of fully virologically suppressive HAART (29).
- Subcutaneous polyethylene glycol (PEG)-modified IL-2 appears to be less biologically active than continuous infusion intravenous IL-2 (CIVIL-2) (30).
- Subcutaneous rIL-2 is better tolerated than CIVIL-2; the magnitude of the CD4+ T-cell count response was proportional to the baseline CD4+ T-cell count (31).
- 5 days of CIVIL-2 are superior to 3-day cycles with respect to CD4+ T-cell expansion (32) but cycle duration longer than 5 days or every 4 week versus every 8 week cycles using CIVIL-2 (30) or subcutaneous rIL-2 were associated with increased adverse effects without additional CD4+ T-cell expansion.
- Subcutaneous rIL-2 7.5 million international units (MIU) induces significantly higher CD4+ T-cell increases than 1.5 MIU, although 4.5 MIU doses are biologically active (33-36).
- CD4+ T-cell count increases can be sustained for up to 5 years without adverse effects on steady state HIV-1 plasma RNA levels and often with reduced dosing cycle frequency (31, 37-40).
- In summary, these phase II studies demonstrated that the cyclical administration of subcutaneous rIL-2 (4.5–7.5 MIU per dose) twice daily for 5 consecutive days every 8 weeks with a background of ART resulted in large increases in CD4+ T-cell count, minimal changes in plasma HIV viral load and no significant changes in CD8+ T-cell count (41).

These early studies set the stage for two large prospective randomized controlled trials: the Subcutaneous Recombinant, Human Interleukin-2 in HIV-Infected Patients with Low CD4+ Counts under Active Antiretroviral Therapy (SILCAAT) study and the Evaluation of Subcutaneous Proleukin in a Randomized International Trial (ESPRIT) study (42). In each, patients infected with HIV who had CD4+ cell counts of either 50 to 299 per cubic millimeter (SILCAAT) or 300 or more per cubic millimeter (ESPRIT) were randomly assigned to receive rIL-2 plus ART or ART alone. The rIL-2 regimen consisted of cycles of 5 consecutive days each, administered at 8-week intervals. The SILCAAT study involved six cycles and a dose of 4.5 million IU of rIL-2 twice daily; ESPRIT involved three cycles and a dose of 7.5 million IU twice daily. Additional cycles were recommended to maintain the CD4+ cell count above predefined target levels. The primary endpoint of both studies was opportunistic disease or death from any cause. In the SILCAAT study, 1695 patients (849 receiving rIL-2plus ART and 846 receiving ART alone) who had a median CD4+ cell count of 202 cells per cubic millimeter were enrolled; in ESPRIT, 4111 patients (2071 receiving rIL-2 plus ART and 2040 receiving ART alone) who had a median CD4+ cell count of 457 cells per cubic millimeter were enrolled. Over a median follow-up period of 7 to 8 years, the CD4+ cell count was higher in the rIL-2 group than in the group receiving ART therapy alone — by 53 and 159 cells per cubic millimeter, on average, in the SILCAAT and ESPRIT studies, respectively. Hazard ratios for opportunistic disease or death from any cause with rIL-2 plus antiretroviral therapy (vs. antiretroviral therapy alone) were 0.91 (95% confidence interval [CI], 0.70 to 1.18; P=0.47) in the SILCAAT study and 0.94 (95% CI, 0.75 to 1.16; P=0.55) in ESPRIT. The hazard ratios for death from any cause and for grade 4 clinical events were 1.06 (P=0.73) and 1.10 (P=0.35), respectively, in the SILCAAT study and 0.90 (P=0.42) and 1.23 (P=0.003), respectively, in ESPRIT (42). Thus, to date, there is no evidence that the administration of IL-2 confers clinical benefit to persons with ART-treated HIV infection.

A.5. PHARMACOLOGIC AND SAFETY PROFILE OF THE STUDY AGENT

Interleukin-2 (IL-2) in its natural form is a 15-Kdalton immunoregulatory protein. Natural IL-2 is hydrophobic and stable at 70°C for 15 minutes at a pH of 2 to 10 in 0.1 percent sodium dodecyl sulphate (SDS) and 6M urea (43, 44). It is produced *in vivo* by T-lymphocytes following antigen activation and is central to the appropriate responsiveness of the immune system challenged by invading pathogens. Its synthesis and release by CD4+ T-lymphocytes, in particular, are of critical importance to the orchestrated events that follow detection of foreign antigen and lead to clearance of pathogen and resolution of disease. Exposure to IL-2 results in the proliferation and maturation of both T- and B-lymphocytes (45). After proliferation, immune system cells retain their antigen specificity. *In vitro*, exogenous IL-2 can restore the natural killer (NK) activity and the cytolytic activity of lymphocytes from patients with AIDS, directed toward CMV-infected target cells (26) as well as against tumor targets.

Recombinant DNA technology has made the production of rIL-2 in large quantities possible. Messenger RNA from the human Jurkat cell line was used to create a double-stranded cDNA that was cloned into pBR322 plasmids; a clone containing the IL-2 gene was identified; the gene was inserted into a region of the plasmid that has a convenient restriction site; the appropriate promoter and ribosome binding site was inserted in front of the IL-2 gene, and the resulting expression clone encoded a modified recombinant IL-2 (rIL-2). Unlike native IL-2, this modified rIL-2 has no N-terminal alanine and has serine substituted for cysteine at amino acid position 125, resulting in a more homogenous IL-2 product (46). The molecule is not glycosylated because it is derived from *Escherichia coli*.

The most prominent side effects of SC rIL-2 occur 2-6 hours following dosing and may be associated with peak plasma levels of rIL-2; most toxicities resolve within 5 days of completion of the treatment cycle, reflecting the short half-life of the agent. At doses used in recent SC rIL-2 HIV trials, toxicities seen have included fever, fatigue, myalgias, and other constitutional symptoms that were dose-dependent and similar to those seen with CIVIL-2 (46). In addition, most patients experienced a local rash characterized by erythema, tenderness, and occasional nodularity at the site of injection.

A syndrome of acute cholecystitis with right upper quadrant pain and nausea and vomiting in HIV-infected patients receiving rIL-2 was described by Powell and coworkers (47). By ultrasound, the gallbladder was noted to be thickened and no calculi were observed. Symptoms resolved with cessation of rIL-2 therapy, and surgery was not required.

Thyroid function abnormalities have been reported in participants receiving IL-2 treatment for malignancies (48-54) and for HIV (19, 33, 37, 42, 55). The reported frequency of thyroid abnormalities among persons receiving IL-2 has varied from 2.5 to 47%, but early reports included participants who received much larger doses than those planned in this study, administered intravenously, and often in combination with other immunomodulatory therapies such as lymphokine-activated killer (LAK) cells (48, 49) or interferon alfa . Among HIV-infected participants receiving subcutaneous rIL-2, the frequency of thyroid dysfunction in small studies has been reported to be between 2.5 and 11.7% (33, 37, 56). The two largest trials of rIL-2 among HIV-infected participants (42), however, did not specifically report the frequency of thyroid dysfunction, but senior investigators in those teams estimate the frequency to be between 10 and 15% (Lane HC, Levy Y, Kovacs J, Sereti I, verbal communications).

The progression of thyroid dysfunction during rIL-2 treatment is variable, but it is thought to involve an initial period of thyroid stimulation akin to autoimmune thyroiditis, which is usually undetected, followed by variable degrees of hypothyroidism (50, 55, 57). The mechanisms of these effects on the thyroid are incompletely understood. It has been proposed that IL-2-mediated induction of proinflammatory cytokine production may trigger the initial autoimmune thyroiditis (48, 50, 51). This notion is supported by the finding of increased lymphocyte infiltration of the thyroid, with features suggestive of autoimmune thyroiditis, during rIL-2 treatment (58, 59). In this model, the initial thyroiditis may result from enhancement of class II expression and presentation of autoantigens by thyrocytes due to increased local release of proinflammatory cytokines by thyroid-infiltrating mononuclear cells (48, 51). The clinical course of rIL-2-induced thyroid dysfunction has not been comprehensively described, but at least in some cases, there is spontaneous resolution of the thyroid abnormalities. In a closely monitored study of a single cycle of rIL-2 administered to 20 euthyroid HIV-infected participants, there was a rapid concomitant rise in TSH, T3, T4, and free T4 within the first three days of treatment, with return of all indices to baseline after approximately 14 days (55). The need for thyroid hormone replacement for rIL-2-induced hypothyroidism has been variable and inconsistently reported. In 3 out of 7 participants who developed hypothyroidism in a trial of rIL-2 given with LAK cells for malignant diseases, thyroid function tests returned to normal while these participants were not receiving thyroid replacement, but the authors indicate that they could not ascertain the course of thyroid dysfunction for other participants (49). In a similar study of cancer patients, thyroid function tests returned to the normal range in the absence of thyroid replacement therapy approximately 6 months after treatment among 4 of the 7 participants with rIL-2-induced hypothyroidism. The remaining 3 participants received levothyroxine and also had normal thyroid tests 6 months after rIL-2 treatment, but it is unclear if thyroid replacement had been discontinued by that time (48). In a study including 49 HIV-infected participants randomized to either low (1.5 MIU

SC BID) or high (7.5 MIU SC BID) dose rIL-2, 2 of the 5 participants who developed hypothyroidism did not receive thyroid replacement therapy, but the duration of thyroid replacement and the follow-up results of thyroid function tests were not reported (33).

Risk factors for thyroid dysfunction during rIL-2 treatment include female sex, pre-existing thyroid dysfunction, large cumulative doses of rIL-2, and importantly, the presence of anti-thyroid antibodies (49, 50, 57). The association of IL-2 thyroid dysfunction with the presence of anti-thyroid antibodies has not been accurately quantified, but it has been observed in multiple reported studies (48, 49, 52). In one study including 34 cancer patients, antithyroid antibodies were present in none of the 27 participants who remained euthyroid during treatment, but were present in 5 out of the 7 (71%) participants who developed hypothyroidism (49, 52). Therefore, in this study, participants will be screened for the presence of anti-thyroid antibodies (in addition to routine thyroid function testing) both at study entry and before each rIL-2 cycle. Participants who have abnormal thyroid function tests or elevated levels of antithyroid antibodies at screening will be excluded. Participants who develop abnormal thyroid function tests or antithyroid antibodies at any time during study participation will be required to discontinue study treatment permanently.

Patients may experience mental status changes when taking rIL-2, including irritability, insomnia, confusion, or depression. Mental status changes due solely to rIL-2 are generally reversible when drug administration is discontinued. However, alterations in mental status may continue for several days before recovery begins.

In ESPRIT, there was an excess of grade 4 clinical events in the rIL-2 recipients; the same excess was not seen in SILCAAT. Abrams and colleagues (42) have suggested this is via an impact on procoagulant pathways. In ESPRIT, patients with the highest CD4+ T-cell counts in the rIL-2 arm had the greatest relative risk of clinical events, including death. Perhaps, therefore, receipt of rIL-2 primes the patient for a proinflammatory state subsequently associated with more clinical events, and this is amplified in patients with the greatest CD4+ T-cell response to rIL-2, i.e. the ESPRIT cohort, which had higher baseline CD4+ T-cell counts than in SILCAAT. However, it is still important to remember that while rIL-2 receipt in ESPRIT was associated with more embolic events, overall there was no excess of opportunistic diseases, death or cardiovascular disease and non-AIDS cancers compared with the ART-only arm.

There is a theoretical risk of emergence of antibodies against IL-2 with exogenous administration, as is the case with other biological products. This appears to be uncommon with rIL-2, however, as neutralizing antibodies were not detected among 77 metastatic renal cell carcinoma patients and 50 metastatic melanoma patients treated with rIL-2. Only 1 out of 106 patients treated with IV rIL-2 for various other indications developed neutralizing antibodies, without associated clinical manifestations. Low titers of non-neutralizing antibodies have been observed in 66 to 74% of patients treated with rIL-2. (Proleukin® Product Monograph, Novartis Pharmaceuticals, revision dated September 16, 2014). Therefore, the probability of emergence of biologically significant anti-IL-2 antibodies in this small study is thought to be extremely low. Nonetheless, immunogenicity testing will be conducted at several points during the study, and participants who have persistent neutralizing antibodies at the end of study visit will be followed until resolution or 12 months after the end of study, whichever is first.

B. HYPOTHESES AND STUDY OBJECTIVES

The purpose of this pilot study is to observe the effects of up to eight 8-weekly cycles of subcutaneous recombinant interleukin-2 (SC rIL-2) on levels of replication-competent HIV in blood CD4 T cells in 20 participants with chronically suppressed HIV infection (viral load <50 copies/mL) who are stable on the same ART regimen for at least one year and have a CD4 T cell count ≥350 cells/mm^3. We hypothesize that IL-2 administration will result in a reduction of the replication-competent reservoir, as measured by the QVOA assay, compared to the baseline values in this population.

B.1. STUDY ENDPOINTS AND OUTCOME MEASURES

The primary endpoint of the study is the change in the level of replication-competent virus from baseline to the end of rIL-2 treatment, as measured by the Quantitative Viral Outgrowth Assay (QVOA).

B.2. PRIMARY OBJECTIVE

The primary objective will be to compare levels of replication competent HIV in circulating CD4 T cells before and after up to 8 cycles of rIL-2 administration. The frequency of CD4 T cells containing replication-competent virus in leukapheresis samples obtained at baseline and at the end of rIL-2 treatment will be enumerated using several state of the art methods. The primary readout for this objective will be measurement of replication-competent virus by Quantitative Viral Outgrowth Assay (QVOA) performed by Robert Siliciano's group at Accelevir.

B.3. SECONDARY OBJECTIVES

1) To examine the relationships between levels of replication competent HIV as identified by QVOA and other measures of HIV persistence:
   a) the intact proviral DNA assay (Siliciano),
   b) the Envelope Detection by Induced Transcription-based Sequencing (EDITS, Karn) performed on the same pre-trial and post-trial leukapheresis samples.
2) To explore the effects of rIL-2 administration on induction of HIV expression in vivo as measured by the EDITS assay and plasma HIV levels.
3) To examine the effects of rIL-2 administration on natural killer cell phenotype and function and on the ability of NK cells to limit HIV propagation in vitro.

C. METHODS

C.1. STUDY DESIGN

This is an open label pilot study of 20 participants with chronically suppressed HIV infection (viral load <50 copies/mL), stable on an ART regimen for at least one year, who have a CD4 T cell count ≥350 cells/mm^3. All participants will receive rIL-2.

All participants will receive rIL-2, 5 million units (MU) subcutaneously (SC) twice a day x 4 consecutive days, every 8 weeks, for 8 cycles in addition to ART.

Ideally, rIL-2 doses should be 10 – 12 hours apart, but no closer than 6 hours, and no greater than 18 hours apart.

If at any point rIL-2 is discontinued, efforts will be made to maintain follow-up for all data collection as outlined in the schedule of events.

C.2. STATISTICAL CONSIDERATIONS

C.2.1. Analytic Plan

The primary analysis will test whether the change in the size of the replication-competent reservoir, as measured by the modified QVOA assay, from baseline to two weeks after the last rIL-2 infusion is significantly different than is expected by random variation. In this intent-to-treat analysis, all participants who received at least one dose of IL-2 will be included. Secondarily, we will perform an on-treatment analysis including only the subset of participants who received all eight planned IL-2 cycles. We will use mixed effects models to estimate the effect of IL-2 treatment on the replication-competent reservoir among all participants, controlling for possible confounding factors including at a minimum CD4 T cell count at baseline and at pre-ART nadir, duration of virologic suppression, type of antiretroviral regimen at study entry, historical peak plasma HIV RNA, and demographic factors.

We will then fit the relevant models to address the following secondary objectives:

a. Replicate the analyses above in the on-treatment population, i.e., those who received all 8 planned IL-2 cycles.

b. In the intent-to-treat population, add a term indicating the number of IL-2 cycles each participant received. This will account for variability of total dose, and the associated parameter and its interaction to time point will provide an estimation of the effect modification expected from repeated dosing. We will assess for non-linearity of any dose effect both graphically and formally, and fit higher-order terms as suggested by those analyses, to explore whether there is a threshold effect (i.e., whether the effect size changes drastically past a given number of doses), an analysis that was not reported in the original Chun paper.

c. Use each of the alternate readouts as the outcome to obtain estimates of the effect size on each of those assays, and then, fit a reverse regression model to estimate the effect of treatment on *any* of the readouts for which a treatment effect is demonstrated. While many approaches are available for this type of problem, reverse regression has the appealing feature for this small-size trial of not being as overly penalizing as more traditional approaches to the multiplicity problem, such as Bonferroni-like corrections (60). We will also use conventional nonparametric correlation analyses and the intraclass correlation coefficient derived from mixed effects models using each of the readouts to assess how the various readouts relate to one another.

d. We will then fit models with time-updated total CD4 T cell count as a covariate. These models will allow us to establish how much of the variation in the change of the measured reservoir size is attributable to the expected CD4 T cell increase due to IL-2 administration. We do not believe that, if the magnitude of the effect is similar to the difference observed between the IL-2 alone and the HAART alone groups in the Chun paper, this can be explained simply by a dilutional effect, but this will be tested more formally in this phase of the analysis.

C.2.2. Power and Sample Size Considerations

We base our sample size calculations on the primary analysis using a mixed effect model to estimate the effect of IL-2 administration on the magnitude of the inducible reservoir by QVOA. In the Chun experiment, the difference between the IL-2 and the HAART-only group was 0.64 IUPM, and the variance in the IL-2 group was 0.41. In a 200-simulation run, we estimate that a total sample size of 20 participants will provide an 80% power (95% CI, 0.74-0.84) to detect a difference from baseline to post-IL-2 administration as large as that seen in the Chun paper. Of note, these estimates are likely conservative, since the variation observed in repeated measurements with the optimized version of the QVOA assay that will be used in this study may be lower (variance around 0.11) than that of the assay used in the original Chun report(2).

C.3. INCLUSION CRITERIA

Participants may enroll in the study if they meet the following criteria:

1. Written informed consent signed and dated by study participant.
2. Male or female, at least 18 years of age and not older than 65 years of age.
3. HIV-1 infection, documented by and FDA-approved ELISA, EIA, or rapid antibody detection method, and confirmed by a second approved antibody-based test or by a positive approved HIV RNA detection assay.
4. CD4+ T cell count ≥ 350 cells/mm^3.
5. HIV-1 RNA < 50 copies/mL obtained within 60 days prior to study entry performed with an FDA-approved HIV-1 RNA assay.
6. Adequate venous access and no other contraindications for leukapheresis.
7. Absolute neutrophil count (ANC) $\geq$ 1500/mm^3.
8. Hemoglobin level >10 g/dL.
9. Platelet count $\geq$100,000/mm^3.

2 Accelevir, data on file

10. Serum creatinine ≤1.5 mg/dL
11. AST and ALT <2.5 times the upper limit of normal
12. TSH, T3, and T4 levels within the normal range of the processing laboratory.
13. Anti-thyrosine peroxidase (TPO) antibodies within the normal range of the processing laboratory.
14. Willing to comply with study-mandated evaluations; including not changing antiretroviral regimen (unless medically indicated) during the study period.
15. All participants must have received HAART, and had viral loads below the limit of quantification of the assay for at least 1 year. Participants who had intermittent isolated episodes of detectable low-level viremia < 500 copies RNA/mL flanked by viral loads below the limit of quantification of the assay will remain eligible.
16. On a stable combination antiretroviral regimen (no changes to treatment within 4 weeks of enrollment) including at least 3 medications and willing to continue on current antiretroviral therapy for the duration of the study, unless otherwise medically indicated.

C.4. EXCLUSION CRITERIA

1. Childbearing potential for female participants. For the purposes of this study, a woman is considered to be of childbearing potential if she is not postmenarchial, has not had a documented surgical sterilization procedure, has an intact uterus and at least 1 ovary, and has had a spontaneous menstrual period in the last 2 years.
2. Acute or chronic hepatitis C infection, defined as a positive plasma HCV RNA using any FDA-approved qualitative or quantitative test in a participant with a positive HCV antibody (HCV RNA testing is not required in participants with a negative HCV antibody). Participants who have completed a course of a direct-acting antiviral agent for hepatitis C and have a confirmed plasma HCV RNA level below the limit of detection of the assay 12 weeks or longer after completion of therapy will be eligible.
3. Acute or chronic hepatitis B infection, defined as a positive HBV surface antigen or a positive HBV DNA.
4. History of advanced chronic liver disease, including cirrhosis, advanced liver fibrosis, severe portal hypertension, or manifestations or hepatic failure.
5. History of malignant disease that is not considered to be surgically or medically eradicated or that has required any form of therapy in the past 5 years.
6. Current diagnosis of congestive heart failure of any severity, uncontrolled angina or uncontrolled arrhythmias.
7. History of lung disease that has required pharmacologic treatment, oxygen supplementation, medical monitoring, or hospitalization in the previous year, or that is expected to cause persistent or recurrent pulmonary symptoms or impairment. Examples of the latter include but are not limited to chronic bronchitis, emphysema, and pulmonary fibrosis.
8. History or any features on physical examination indicative of a bleeding diathesis.
9. History or current diagnosis of thromboembolic disease, including deep vein thrombosis and pulmonary embolism, or family history of the same.
10. History of hypersensitivity to radiological contrast media or anticipated need for exposure to radiological contrast media during the study period.
11. Use of chronic corticosteroids, hydroxyurea, or immune-modulating agents (e.g., interleukin-2, interferon-alpha or gamma, granulocyte colony stimulating factors, etc.) within 30 days prior to enrollment.
    NOTE: Use of inhaled or topical steroids is not exclusionary.
12. Breast-feeding.

13. Use of aspirin, warfarin or any other antithrombotic or antiplatelet agent during the 2-week period prior to leukapheresis.
14. History of autoimmune disorders, including but not limited to Crohn's disease, scleroderma, thyroiditis, inflammatory arthritis, myasthenia gravis, glomerulonephritis, systemic lupus erythematous, and vasculitis.
15. Type 1 diabetes mellitus.
16. History of thyroid disease requiring that has required antithyroid or thyroid hormone replacement therapy at any time in the past.
17. Current continued use, or anticipated continued medical indication for nephrotoxic agents, including but not limited to aminoglycosides and other potentially nephrotoxic antimicrobials, indomethacin, high scheduled doses of other NSAIDs, and lithium salts.
    NOTE: Low doses or limited duration of these agents (i.e., ≤14 days) is not exclusionary.
18. Current continued use, or anticipated continued medical indication for hepatotoxic agents, including but not limited to amiodarone, methotrexate, and anticonvulsants and antimicrobials with elevated hepatotoxic potential.
    NOTE: Low doses or limited duration of these agents (i.e., ≤14 days) is not exclusionary.
19. Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.
20. Serious illness requiring systemic treatment and/or hospitalization within 30 days prior to study entry that in the judgement of the investigator may compromise study participation or pose additional risks to the participant.
21. Any other condition that, in the opinion of the clinical investigator or sponsor, might compromise any aspect of this trial.

C.5. STUDY TREATMENT PLAN

C.5.1. Study Medication (rIL-2)

Proleukin® (Prometheus Laboratories) is provided as a sterile, white to off-white, lyophilized cake in glass vials, each containing 22 MIU.

C.5.1.1. Dosage and Cycle Guidelines

Participants will receive rIL-2 at an initial dose of approximately 5 MIU twice daily for 4 consecutive days every 8 weeks (8 cycles total). Each vial will be reconstituted according to the package insert instructions using 1.2 mL of diluent, and 4 doses will be obtained from each vial. Occasionally, the reconstituted volume that can be drawn from the vial may result in doses that are slightly smaller than 5 MIU each. These small deviations from the total intended dose are biologically inconsequential and do not constitute protocol deviations.

C.5.1.2. Preparation of rIL-2 for Subcutaneous Administration

The Investigational Pharmacy will reconstitute rIL-2 at the beginning of each cycle for each participant, and draw each individual dose into tuberculin syringes under sterile conditions. As was done in two previous large trials of rIL-2 in HIV-infected participants(19), all eight doses will be prepared, labeled, and delivered to the participant on day 0 of each cycle. This procedure may be modified in the course of

the study if deemed necessary by the Investigational Pharmacy, the investigators, or a regulatory authority, including the option to prepare a smaller number of doses in advance. In that case, pre-filled syringes may be shipped by fast courier to the participant's residence.

Participants will be taught to give themselves rIL-2 injections. This will require substantial clinician support in terms of both time spent instructing participants and in availability to answer participant questions and respond to participant concerns about injections and side effects. Refer to the Manual of Operating Procedures (MOPS) document for further details on initial and subsequent dose administration

C.5.2. Guidelines for Dosage Modification

Participants will be monitored closely for side effects of rIL-2. Participants who experience dose-limiting side effects may have their rIL-2 temporarily interrupted or their dose reduced.

The Manual of Operations (MOPs) includes a list of toxicities for which the investigator may choose to temporarily interrupt or dose-reduce rIL-2 (hereinafter, dose-limiting toxicities). An outline of criteria for certain specific potential adverse events are provided in section E.1. Briefly, the following guidelines apply:

- For toxicities during the 4-consecutive-day administration of rIL-2 that are considered dose-limiting, the remaining rIL-2 doses of that cycle of therapy will be interrupted until the dose-limiting toxicity is no longer consider dose-limiting.

- In some circumstances, a toxicity may be regarded as dose-limiting in the opinion of the investigator, even if it does not meet the above-mentioned criteria. In these instances, the clinical judgment of the investigator takes precedence.

- When rIL-2 treatment is reinitiated after interruption because of a dose-limiting toxicity, the dose will be reduced by up to 1.5 MIU per injection (3 MIU/day). If the reduced dose results in a dose-limiting toxicity during the same cycle, rIL-2 therapy will again be interrupted. Further dose reduction, if necessary, will be made in the same fashion.

- If a participant cannot tolerate an rIL-2 dose of 2 MIU twice a day then rIL-2 therapy will be permanently stopped.

- If, after a dose reduction, a participant completes a treatment cycle without significant toxicity, then escalation of the rIL-2 dose at the next cycle may occur by an increment of up to 1.5 MIU/dose (3 MIU/day).

- The total duration of a course of rIL-2 will not be extended for doses missed as a result of toxicity or any other reason.

- If a participant develops a toxicity that is subjectively intolerable, but which does not meet criteria for a dose-limiting toxicity, the dose of rIL-2 can be lowered after consultation with the protocol chairs (employing the above-described dose decrement

scheme) without actually interrupting the treatment phase of the cycle.

- Toxicities that result in a dose reduction or interruption will be recorded on the CRF.

C.6. CONCOMITANT MEDICATIONS

All participants should be on an HAART regimen for at least one year before enrollment, without changes in the 4 weeks prior to enrollment. Changes in HAART regimen after enrollment are to be undertaken at the discretion of the treating physician and as clinically indicated, but should otherwise be avoided.

Symptomatic treatment for management of expected clinical manifestations associated with IL-2 treatment is permitted according to the guidelines contained in the MOPS.

Co-administration of cytotoxic agents with rIL-2 is prohibited, as is the administration of any medications that meet exclusion criteria.

Recombinant IL-2 may affect central nervous system function. Thus, interactions could occur following concomitant administration of psychotropic drugs (e.g., narcotics, analgesics, antiemetics, sedatives, antidepressants and tranquilizers). The investigators will determine whether the clinically indicated use of any of the prohibited medications requires discontinuation of study treatment.

Although systemic use of glucocorticoids has been shown to reduce IL-2-induced side effects, including fever, concomitant administration of these agents with rIL-2 may reduce the effectiveness of rIL-2; therefore, they will not be used for symptomatic management. If there is a clinical indication for use of systemic steroids after enrollment, however, this will be recorded in the concomitant medications log, but will not require discontinuation of study treatment.

Beta-blockers and other antihypertensives may intensify the hypotension seen with rIL-2. Thus, participants receiving these medications will be enrolled only after careful review of the doses and combinations of antihypertensive medications they are receiving by the investigators.

D. CLINICAL AND LABORATORY EVALUATIONS

D.1. SCHEDULE OF EVENTS

Refer to the Manual of Operations (MOPS) for the schedule of events chart.

D.2. TIMING AND CONTENT OF EVALUATIONS

D.2.1. Screening Visit

The screening visit will occur 8-16 weeks prior to start of rIL-2 treatment, and will include:

- History and complete physical examination that includes evaluation of adequate venous access for leukapheresis
- Height and weight measurements
- Concomitant medication review
- CBC and metabolic panel
- Pregnancy test
- Thyroid function tests and anti-Tpo antibodies
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes).
- Plasma HIV RNA level (using the locally available assay) and verification that plasma HIV RNA levels prior to screening meet the inclusion criteria.
- Hepatitis B and C testing
- Informed consent

D.2.2. Leukapheresis Visit

The pre-treatment leukapheresis visit will occur 4-8 weeks prior to start of rIL-2 treatment, and will include:

- Interval history and targeted physical examination
- Concomitant medication review.
- CBC and metabolic panel
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes) and documentation of all interim CD4+ counts (absolute and percents).
- Thyroid function tests and anti-Tpo antibodies.
- Participant will be instructed as to how IL-2 will be injected subcutaneously. Instructions for preparing rIL-2 for administration are given in the Manual of Operations, which includes the Investigator's Brochure as an appendix. Participants receiving rIL-2 will be taught to give themselves injections. Participants will be given a dosing log for future use and will be encouraged to refer to it during their daily activities. Supporting materials for clinical staff and for participants on administering rIL-2 and managing expected side effects are included in the Manual of Operations. The Manual of Operations also includes contact information for questions concerning clinical management. No rIL-2 will be administered at this visit.
- A 4 liter leukapheresis will be performed at the Seidman Ambulatory Stem Cell Transplant

Unit at University Hospitals using AIDS protocol 328.

- Plasma will be frozen for single copy HIV RNA assay.
- Leukapheresis samples will be processed into PBMC that will be cryopreserved for assays of:
  - HIV reservoir by QVOA
  - HIV reservoir by EDITS
  - Total and intact proviral DNA assay (Accelavir)
  - Immune Cell Phenotype
  - NK cell phenotype

D.2.3. rIL-2 Initial Treatment Visit – Cycle 1, Day 0

The first IL-2 cycle will start 4-8 weeks after the leukapheresis visit and will include:

- Interval history and targeted physical examination, including weight.
- Concomitant medication review.
- Delivery of pre-filled cycle doses of rIL-2 and review of handling, storage, and administration procedures
- CBC and metabolic panel
- Thyroid function tests and anti-Tpo antibodies
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes)
- Plasma HIV RNA level by PCR (using the locally available assay)
- IL-2 immunogenicity
- PBMC will be cryopreserved for assays of immune cell phenotype and NK cell phenotype
- Plasma will be cryopreserved for single copy HIV RNA assay
- The participant will be instructed again as to how IL-2 will be injected subcutaneously. Instructions for preparing rIL-2 for administration are given in the Manual of Operations, which includes the Investigator's Brochure as an appendix. Participants receiving rIL-2 will be taught to give themselves injections. Questions regarding the use of the dosing log will be resolved at this time. Supporting materials for clinical staff and for participants on administering rIL-2 and managing expected side effects are included in the Manual of Operations. The Manual of Operations also includes contact information for questions concerning clinical management.
- The participant will be observed as he/she administers the first dose of rIL-2 (approximately 5 million units) subcutaneously, and this dose will be recorded, under the supervision of the research staff conducting the visit, on the dosing log.
- The participant will be observed for approximately 4 hours after the injection. During the administration period, vital signs will be monitored every hour.

D.2.4. rIL-2 Treatment Cycle 1, Day 7

Participant returns to clinic for:

- Interval history and complete physical examination.
- Concomitant medication review
- Tolerance and compliance with injection schedule
- CBC and metabolic panel

- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes)
- Plasma HIV RNA level by PCR (using locally available assay)
- IL-2 immunogenicity
- PBMC will be cryopreserved for HIV reservoir assay by EDITS
- PBMC will be cryopreserved for assays of immune cell phenotype and NK cell phenotype
- Plasma will be cryopreserved for single copy HIV RNA assay

D.2.5. rIL-2 Treatment Cycles 4 and 8, Day 0

NOTE: Each cycle should start 7-10 weeks after day 0 of the last treatment cycle. These visits will include:

- Interval history and targeted physical examination, including weight.
- Concomitant medication review.
- Delivery of pre-filled cycle doses of rIL-2 and review of handling, storage, and administration procedures
- CBC and metabolic panel
- Thyroid function tests and anti-Tpo antibodies
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes)
- Plasma HIV RNA level by PCR (using the locally available assay)
- PBMC will be cryopreserved for assays of immune cell phenotype and NK cell phenotype
- Plasma will be cryopreserved for single copy HIV RNA assay
- The participant will be observed as he/she administers the dose of rIL-2 (approximately 5 million units) subcutaneously.

D.2.6. rIL-2 Treatment Cycles 4 and 8, Day 7

Participant returns to clinic for:

- Interval history and complete physical examination.
- Concomitant medication review
- Tolerance and compliance with injection schedule
- CBC and metabolic panel
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes)
- Plasma HIV RNA level by PCR (using locally available assay)
- PBMC will be cryopreserved for assays of immune cell phenotype and NK cell phenotype
- Plasma will be cryopreserved for single copy HIV RNA assay

D.2.7. rIL-2 Treatment Cycles 2 and 3, Day 0

NOTE: Each treatment cycle should begin 7-10 weeks after start of last treatment cycle. These visits will include:

- Interval history and targeted physical examination, including weight.
- Concomitant medication review.
- Delivery of pre-filled cycle doses of rIL-2 and review of handling, storage, and administration procedures

- CBC and metabolic panel
- Thyroid function tests and anti-Tpo antibodies
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes)
- Plasma HIV RNA level by PCR (using the locally available assay)
- IL-2 immunogenicity (cycle 2 only)

D.2.8. rIL-2 Treatment Cycles 5, 6, and 7, Day 0

NOTE: Each treatment cycle should begin 7-10 weeks after start of last treatment cycle. These visits will include:

- Interval history and targeted physical examination, including weight.
- Concomitant medication review.
- Delivery of pre-filled cycle doses of rIL-2 and review of handling, storage, and administration procedures
- CBC and metabolic panel
- Thyroid function tests and anti-Tpo antibodies
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes)
- Plasma HIV RNA level by PCR (using the locally available assay)

D.2.9. rIL-2 Treatment cycles 1-8, Days 2 and 4

Staff will contact the participant by phone to assess tolerance and compliance with injection schedule

D.2.10. End of Study Visit (or Premature Discontinuation Visit)

NOTE: The end of study visit should take place 8 weeks (+/- 2 weeks) after Day 0 of the last (8^{th}) treatment cycle. In the case of premature discontinuation, the end of study visit should take place at least 6 weeks after day 0 of the last treatment cycle and may take place up to 10 weeks after day 0 of the last treatment cycle.

These visits will include:

- Interval history and targeted physical examination.
- Immunogenicity testing
- CD4+ and CD8+ T cell count (both absolute and as percent of lymphocytes)
- Plasma HIV RNA level by PCR (using the locally available assay)
- IL-2 immunogenicity
- At this visit the participant will undergo a 4 Liter leukapheresis at the Seidman Ambulatory Stem Cell Transplant Unit at University Hospitals using AIDS protocol 328.
- Plasma will be cryopreserved for single copy HIV RNA assay
- Leukapheresis product PBMC will be cryopreserved for:
  - HIV reservoir assay by EDITS
  - HIV reservoir assay by QVOA
  - Total and intact proviral DNA assay (Accelavir)
  - Assays of immune cell phenotype and NK cell phenotype

If leukapheresis cannot be done after premature treatment discontinuation for any reason, a 120 cc. blood draw will be obtained for preparation of PBMC and plasma. Based upon cell yield these samples will be used for:

- Plasma will be cryopreserved for single copy HIV RNA assay
- HIV reservoir assay by EDITS
- HIV reservoir assay by QVOA
- Total and intact proviral DNA assay (Accelavir)
- Assays of immune cell phenotype and NK cell phenotype

D.2.11. Immunogenicity follow-up

Participants who have detectable neutralizing antibodies at the end of study visit will be followed approximately every 90 days for repeat testing until 12 months after the end of study or until a negative result is obtained. During these visits, an interval medical history and targeted physical exam will be conducted. If clinically significant events have occurred, the participant will be referred for appropriate medical care.

D.2.12. Management of off-window visits

Every effort should be made to conduct each visit within its allocated window. If, however, a participant cannot conduct a scheduled visit within the scheduled window, the principal investigators will determine whether the visit can be conducted within a few days outside of the per-protocol window. If conducting the visit on the proposed off-window date would raise a safety concern or could compromise the integrity of the study, the visit will not be rescheduled and will be recorded as missed. In either case, the reasons for the off-window visit will be recorded in the source document and the protocol deviation CRF will be completed.

D.2.13. Holding rIL-2 cycles

The initiation of an rIL-2 cycle can be delayed or cancelled by the principal investigators, if deemed necessary for clinical or logistical reasons. When a cycle is delayed beyond the end of the scheduled window, the reasons for the delay or cancellation will be recorded in the source document and the protocol deviation CRF will be completed. If a cycle is cancelled, the corresponding day 2, 4, and 7 visits will also be cancelled.

D.3. INSTRUCTIONS FOR EVALUATIONS

All clinical and laboratory information required by this protocol is to be present in the source documents. Results of all approved laboratory tests performed as part of the study will be provided to the participants' physicians, and any resulting clinical action will be taken by the participant and their physician without interference from the study personnel.

D.3.1. Documentation of HIV

For the purposes of this protocol, any combination of two positive results of the following tests is sufficient for documentation of HIV infection: a) FDA-approved HIV ELISA or EIA; b) FDA-

approved HIV Western blot; c) FDA-approved HIV rapid test; d) p24 antigen immunoassay; e) FDA-approved nucleic acid test; f) detectable HIV RNA in plasma by an FDA-approved method.

D.3.2. Medical History

The medical history must include all diagnoses that were either established within 45 days prior to or ongoing at the time of enrollment. Any allergies to any study medications and their formulations must be documented. All elements of the medical history will be recorded on the source documents.

D.3.3. Medication History

A medication history must be present, including start and stop dates. The table below lists the medications that must be included in the history to be recorded in the source documents.

Table 1. Medications to be recorded

Medication Category	Timeframe
Antiretroviral medications	Complete history
Immune-based therapies	Within 90 days prior to study entry
Vaccines	Within 30 days prior to study entry
All other prescription and non-prescription medications	Within 90 days prior to entry

D.3.4. Clinical Assessments

D.3.4.1. Complete Physical Exam

A complete physical exam is to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the heart and lungs; abdominal exam; and examination of the lower extremities for edema. The complete physical exam will also include vital signs (temperature, heart rate, respiration rate, and blood pressure).

D.3.4.2. Targeted Physical Exam

A targeted physical exam is directed to the site or organ system involved in any previously identified or new signs or symptoms that the participant has experienced since the last visit. This exam also includes vital signs (temperature, heart rate, respiration rate, and blood pressure).

D.3.4.3. Height

Height without shoes in centimeters will be obtained at screening and recorded on the CRF at entry.

D.3.4.4. Weight

Weight without shoes in kilograms will be obtained at screening, leukapheresis visit, at

entry and at day 0 of each cycle. The weight obtained at entry and at day 0 of each cycle will be recorded on the CRF.

D.3.4.5. Interval History

At entry, all signs and symptoms, regardless of grade, that occurred within 45 days before entry will be recorded in the source documents. Post-entry, only grade ≥ 2 signs and symptoms, or those that led to a change in treatment, regardless of grade, will be recorded on the corresponding CRFs.

D.3.4.6. Diagnoses

After study entry, all new diagnoses established while on study will be recorded on the source document. Any gradable diagnoses that meet AE criteria will be recorded on the CRF, and relationship to study agent will be assessed.

D.3.4.7. Concomitant Medications

Concomitant prescription or over-the counter medications initiated or discontinued since the last visit will be recorded on the source documents.

D.3.4.8. Study Treatment Modifications

All study drug modifications, including actually administered doses preceding each of the scheduled in-person or phone assessments will be recorded. Any permanent discontinuation of treatment will be recorded on the CRF.

D.3.4.9. Antiretroviral Medications

All modifications to antiretroviral medications post-entry will be recorded on the CRF. If the modification includes discontinuation of antiretrovirals, the rules for treatment discontinuation (section F.3) must be followed.

D.3.5. Laboratory Evaluations

All clinical laboratory results will be stored as part of the source document. Only emerging (i.e. not present before or at the time of entry) laboratory values grade 2 or higher must be recorded on the CRF.

D.3.5.1. Clinical Laboratory Tests

All clinical labs will be processed in the CLIA-certified clinical laboratory of University Hospitals Cleveland Medical Center. Clinical laboratories include:

- CBC will include hemoglobin, hematocrit, white cell count with differential and platelet count. This test will be submitted and results obtained in real time.
- Metabolic panel will include at least glucose, BUN, creatinine, sodium, potassium, chloride, bicarbonate, total and direct bilirubin, AST, ALT, and alkaline phosphatase.

This test will be submitted and results obtained in real time.

- HIV-1 RNA testing will be done using an FDA-approved method with a lower limit of detection of 50 copies/mL or lower. This test will be submitted in real time, but the turnaround time is approximately 3 days.
- HBsAg with reflex HBV DNA, and HCV Ab with reflex HCV RNA will be performed using FDA-approved kits as needed to determine eligibility. These tests will be submitted in real time. Results will be obtained, however, prior to study entry.
- Thyroid function tests will include TSH, T4, and T3 serum levels.

D.3.5.2. Research Evaluations

Specimens for immunologic monitoring, virologic testing, and storage for deferred assays will be processed, stored, and distributed by Dr. Lederman's laboratory as needed. Immunogenicity testing for antibodies against rIL-2 will be done in batched serum using standard methods for both binding and neutralizing activity. These methods will be validated in Dr. Lederman's laboratory before testing of study-derived samples. Refer to the LPC for details on volumes, collections procedures, transport, and processing.

D.4. SPECIMEN COLLECTION, HANDLING, AND PROCESSING

D.4.1. General Specimen Management Guidance

The study site will adhere to the standards of good clinical laboratory practice, DAIDS Laboratory Requirements and site standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens at the local laboratories, as described in (http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/gclp.pdf)

Specimen collection, testing, and storage at the site laboratories will be documented per standard site practice. In cases where laboratory results are not available due to administrative or laboratory error, specimens intended may be re-drawn if within a window of the intended visit that the investigators determine is acceptable for the scientific goals of the study.

D.4.2. Storage of Specimens for Future Use

Study staff will store all specimens collected in this study on site at least through the end of the study. Specimens will not be labeled with any personal identifiers. Storage of all study samples will follow local standard operating procedure to ensure the anonymity and confidentiality of the trial research participants. Specimens remaining at the end of the study will be transferred to a designated bio-repository with appropriate participants' permission and after all protocol-required and quality assurance testing has been completed. If such permission is not obtained, those participant's samples will be destroyed.

Informed consent for storage of specimens for future studies will be obtained through an existing IRB-approved protocol that governs the existing bio-repository at UHCMC. This protocol exists under the oversight of the Case Western Reserve University Center for AIDS

Research (CFAR), and is one of the resources of the CFAR Clinical Services Core, which is directed by Dr. Lederman. The Clinical Services Core maintains a fully HIPAA-complaint repository management system, and the protocol provides for separate consents to use stored specimens for: a) use of de-identified data for research purposes; b) non-genetic studies; c) genetic studies; and d) authorization to contact participants for future studies. Participants can consent to any, all, or none of the four activities. The repository management system permits tracking and updating in real time, for each record in the database and for each specimen, whether a valid consent of any of the four types described above exists in the system.

E. CLINICAL MANAGEMENT ISSUES

If at any time, a decision is made to discontinue study agent in all participants, the IND Sponsor (Dr. Benigno Rodriguez), after consultation with the protocol team, will inform the US Food and Drug Administration (FDA), the IRB, and the site investigators.

E.1. MANAGEMENT OF SPECIFIC ADVERSE EVENTS

The Guidelines for Dose Modification, (protocol section C.5.2), apply to all of the scenarios listed in this section.

E.1.1. Serious Infections

All subjects will be monitored for signs or symptoms of a serious or local infection at each visit. All subjects who develop a serious infection while on study will be referred for immediate medical care to a qualified healthcare professional. Participants who develop any of the following infectious events will be required to discontinue study treatment:

- Tuberculosis, pulmonary or extrapulmonary
- Non-tuberculous mycobacterial infection, disseminated or extrapulmonary
- *Pneumocystis jirovecii* pneumonia
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis or isosporiasis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or lymph nodes) and including retinitis with loss of vision
- Histoplasmosis, disseminated or extrapulmonary
- Kaposi's sarcoma
- Lymphoma, Burkitt's or immunoblastic, regardless of anatomical location
- Lymphoma, primary of brain
- Progressive multifocal leukoencephalopathy
- Toxoplasmosis of brain
- Herpes zoster reactivation involving more than one dermatome or visceral involvement including but not limited to viral pneumonia, encephalitis and hepatitis.
- Any serious infection that is considered life-threatening or requires inpatient treatment. Participants who experience other serious infections not listed above that do not require inpatient treatment and are not considered life-threatening will have study treatment temporarily stopped until the infection is controlled. These subjects may restart study drug once the infection is controlled.

Other infections, including local infections, will also require referral to qualified medical treatment. The study principal investigator, in consultation with the SMC if necessary, will determine if an infection not listed above warrants permanent treatment discontinuation.

E.1.2. Acute Hypersensitivity Reactions

To minimize the risk of a severe, immediate-type hypersensitivity reaction or capillary leak syndrome, the first dose of study agent will be administered in the Special Immunology Unit by personnel with experience administering rIL-2 to over 50 participants in the SILCAAT and ACTG 328 trials. Participants will be monitored for a minimum of 30 minutes following the first dose of study drug by personnel with life support training and access to adequate life support equipment and resuscitation medication.

Development of a grade ≥3 hypersensitivity reaction, a grade 2 hypersensitivity reaction that includes manifestations of angioedema, or suspected or confirmed capillary leak syndrome will require permanent treatment discontinuation.

E.1.3. Neutropenia

Participants who develop neutropenia will be referred for treatment by a qualified healthcare professional. Volunteers with an ANC <2,000 cells/mm^3 at screening will be excluded.

- Should the ANC decrease to ≥500 but ≤1,000 cells/mm^3, the rIL-2 dose will be interrupted until the ANC is >1,000 cells/mm^3 and then rIL-2 will be restarted at 3.5 million units twice daily. If the ANC then remains above 1,000 cells/mm^3, this dose will be continued until the end of study.
- If the ANC drops to ≥500 but ≤1,000 cells/mm^3 while on the reduced dose of 3.5 million units twice daily, rIL2 will be discontinued until the ANC reaches 1,000 cells/mm^3, and then resumed at a dose of 2 million units twice daily. If the ANC then remains above 1,000 cells/mm^3, this dose will be continued until the end of study. If the ANC falls again to ≤1,000 cells/mm^3 while on the reduced 2 million unit twice daily dose, rIL2 will be permanently discontinued.
- Should the ANC decrease to <500 cells/mm^3, rIL-2 will be permanently discontinued.

E.1.4. Thrombocytopenia

Participants who develop thrombocytopenia will be referred for treatment by a qualified healthcare professional. Volunteers with platelet counts <150,000/mm^3 will be excluded.

- Should the platelet count decrease to ≥50,000 but ≤100,000/mm^3 the rIL-2 dose will be interrupted until the platelet count is >100,000/mm^3 and then rIL-2 will be restarted at 3.5 million units twice daily. If the platelet count then remains above 100,000 /mm^3, this dose will be continued until the end of study.
- If the platelet count drops to ≥50,0000 but ≤100,000/mm^3 while on the reduced dose of 3.5 million units twice daily, rIL2 will be discontinued until the platelet count reaches 100,000/mm^3 and then resumed at a dose of 2 million units twice daily. If the platelet count then remains above 100,000 /mm^3, this dose will be continued until the end of study. If the platelet count falls again to ≤100,000 /mm^3 while on the reduced 2 million unit twice daily dose, rIL2 will be permanently discontinued.
- Should the platelet count decrease to <50,000/mm^3, rIL-2 will be permanently discontinued.

E.1.5. AST and ALT Abnormalities

Participants who develop AST or ALT elevations will be referred for treatment by a qualified healthcare professional. Volunteers with AST or ALT levels > 2.5x ULN at screening will be excluded.

- Volunteers with cirrhosis, severe liver disease, or active hepatitis B or C infection will be excluded from the study.
- Should AST or ALT values rise to > 3x ULN, rIL-2 will be interrupted. rIL-2 may be resumed at 3.5 million units twice daily when AST or ALT fall to < 2x ULN. If AST and ALT values then remain below 3x ULN, this dose will be continued to the end of study.
- If AST or ALT values rise again to >3x ULN while on the reduced dose of 3.5 million units twice daily, rIL-2 will be interrupted until AST and ALT fall to <2x ULN and then resumed at a dose of 2 million units twice daily. If AST and ALT values then remain below 3x ULN, this dose will be continued to the end of study. If AST or ALT levels rise again to >3x ULN while on the reduced 2 million unit twice daily dose, rIL2 will be permanently discontinued.
- If ALT values rise again to > 3x ULN while the patient is receiving 2 million units twice daily, rIL-2 will be discontinued permanently.
- Should AST or ALT values rise to >5x ULN, rIL2 will be permanently discontinued.

E.1.6. Thyroid Test Abnormalities

Participants who develop thyroid function test (TFT) abnormalities will be referred for treatment by a qualified healthcare professional. Volunteers with abnormal TFTs or levels of anti-thyroid antibodies (anti-Tpo) at screening will be excluded.

- If a participant develops a TSH level >3.98 mIU/L, rIL-2 will be permanently discontinued.
- If T3 or T4 levels are outside the normal reference range of the laboratory, rIL-2 will be permanently discontinued.
- If a participant develops anti-Tpo antibody levels that are above the normal reference range of the laboratory, rIL-2 will be permanently discontinued.
- If a participant develops clinical signs or symptoms of hypo- or hyperthryroidism, rIL-2 will be permanently discontinued.

E.1.7. Renal Dysfunction

Participants who develop evidence of renal dysfunction will be referred for treatment by a qualified healthcare professional. Volunteers with serum creatinine >1.5 mg/dL at screening will be excluded.

- Should serum creatinine increase to >2.0 mg/dL but ≤3 mg/dL, rIL-2 will be interrupted. rIL-2 may be resumed at 3.5 million units twice daily when creatinine falls to <1.5 mg/dL. If creatinine remains ≤1.5 mg/dL on the reduced dose of 3.5 million units twice daily, this dose will be continued to the end of study.
- If serum creatinine rises again to >1.5 mg/dL while on the reduced dose of 3.5 million units twice daily, rIL-2 will be interrupted until it falls to <1.5 mg/dL and then resumed at a dose of 2 million units twice daily. If serum creatinine then remains below 1.5 mg/dl, this dose

will be continued to the end of study. If serum creatinine rises again to >1.5 mg/dL while on the reduced 2 million unit twice daily dose, rIL2 will be permanently discontinued.

- Should serum creatinine rise to >3 mg/dL, rIL-2 will be discontinued permanently

E.1.8. Malignant Conditions

All participants will be monitored for signs or symptoms of a malignant condition at each visit, and referred for treatment by a qualified healthcare provider if evidence of malignancy is found. Volunteers with a history of malignant disease that has not been fully resected and considered cured or in remission for at least 5 years will be excluded. Participants who develop a malignancy other than a basal cell skin carcinoma requiring no more than local excision during study will be required to discontinue study treatment permanently.

E.1.9. Viral Reactivation

Participants who develop pain, a skin rash, or other clinical manifestation deemed consistent with an episode of herpes zoster, will be immediately referred for evaluation by a qualified clinician, and will be required to discontinue study treatment until any pharmacologic treatment for varicella-zoster has been completed and the clinical manifestations have resolved in the judgment of the treating physician. Participants who develop recurrence of signs or symptoms or herpes zoster reactivation, and those who develop involvement of more than two dermatomes or have evidence of visceral involvement while on study will be required to discontinue study treatment permanently.

Participants who develop a detectable HBV DNA at any time during the study will be required to discontinue study treatment permanently, and will be referred for specialized medical care a soon as the study team becomes aware of a positive HBV DNA result.

E.1.10. Thrombotic Disease

At each visit, signs and symptoms of venous thrombosis will be sought. Participants who develop deep vein thrombosis or pulmonary embolism will permanently discontinue rIL-2 administration.

E.2. MANAGEMENT OF ADVERSE EVENTS ACCORDING TO SEVERITY

E.2.1. Grade 1 or 2 AEs

Participants who develop a Grade 1 or 2 AE may continue study treatment.

E.2.2. Grade 3 or 4 AEs

If the investigators have compelling evidence that the grade 3 or 4 AE has NOT been caused by the study treatment, dosing may continue without modification. In all other cases, the guidelines for dose adjustment will be applied. Refer to sections E.1 and C.6.2 and to Manual of Operations for dosing modification guidelines.

Participants experiencing Grade 3 or 4 AEs will be followed closely for resolution of the AE to

Grade ≤ 2.

E.3. PREGNANCY

Women of reproductive potential will be excluded from enrollment in the study. In the unlikely event that a participant does become pregnant while on study treatment (e.g., tubal ligation failure), study treatment will be permanently discontinued and the participant will be referred for immediate medical evaluation. Any such participants will be encouraged to remain in the study to be followed on study/off study treatment until study completion and will be followed by telephone contact thereafter to determine the pregnancy outcome. Pregnancy-related outcomes (health of the infant) and any pregnancy-related complications (e.g., fetal loss/abnormalities) will be reported on the CRF.

NOTE: Pregnant participants will be considered on study until the outcome of the pregnancy has been obtained and reported on the CRF.

E.4. REPORTING OF ADVERSE REACTIONS TO THE RESPONSIBLE IRBs

The investigators will report adverse reactions to the responsible IRB in accordance with respective IRB policies and procedures.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the investigator's follow-up investigation show that an adverse event that was initially determined not to require reporting to the IRB does, in fact, meet the requirements for reporting, the investigator will report it as soon as possible in accordance with respective IRB policies and procedures.

E.5. SOCIAL HARMS REPORTING

Although the study site makes every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-infected). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities.

Social harms that are judged by the study chairs to be serious or unexpected will be reported to responsible site IRB at least annually or according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed.

F. CRITERIA FOR DISCONTINUATION

F.1. PERMANENT TREATMENT DISCONTINUATION

- Interruption of antiretroviral medications for >4 consecutive weeks
- Occurrence of any of the following opportunistic complications:

    - Tuberculosis, pulmonary or extrapulmonary
    - Non-tuberculous mycobacterial infection, disseminated or extrapulmonary
    - *Pneumocystis jirovecii* pneumonia
    - Coccidioidomycosis, disseminated or extrapulmonary
    - Cryptococcosis, extrapulmonary
    - Cryptosporidiosis or isosporiasis, chronic intestinal (greater than 1 month's duration)
    - Cytomegalovirus disease (other than liver, spleen, or lymph nodes) and including retinitis with loss of vision
    - Histoplasmosis, disseminated or extrapulmonary
    - Kaposi's sarcoma
    - Lymphoma, Burkitt's or immunoblastic, regardless of anatomical location
    - Lymphoma, primary of brain
    - Progressive multifocal leukoencephalopathy
    - Toxoplasmosis of brain

- Occurrence of herpes zoster reactivation involving more than two dermatomes or visceral involvement including but not limited to viral pneumonia, encephalitis and hepatitis.
- Occurrence of a serious infection that is considered life-threatening or requires inpatient treatment.
- Occurrence of an incident cancer other than a localized basal cell carcinoma of the skin that requires local excision only.
- Requirement for prohibited concomitant medications (see exclusion criteria and section C.6).
- Pregnancy or breastfeeding.
- Deep vein thrombosis or pulmonary embolism.
- Myocardial infarction or other acute coronary syndrome.
- Development of a grade ≥3 hypersensitivity reaction, OR a grade 2 hypersensitivity reaction that includes manifestations of angioedema, or suspected or confirmed capillary leak syndrome.
- Development of an ANC <500 cells/mm^3.
- Development of a platelet count <50,000/mm^3.
- Elevation of AST or ALT to >5x ULN.
- Serum TSH elevation to ≥3.99 mIU/L, abnormal serum T4 or T3 levels, abnormal levels of anti-Tpo antibodies, or symptomatic hypo- or hyperthyroidism.
- Completion of treatment as defined in the protocol.
- Request by participant or his/her healthcare providers to terminate treatment.
- Reasons believed to be clinically significant by the investigators, even if not addressed in the adverse event management section of the protocol.

F.2. PREMATURE STUDY DISCONTINUATION

- Failure by the participant to attend 3 consecutive visits.
- Request by participant or his/her healthcare providers to terminate participation in the study.
- Participant judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the institutional review board (IRB), Office for Human Research Protections (OHRP), FDA, NIAID or investigator.

F.3. TEMPORARY TREATMENT DISCONTINUATION

- Criteria for withholding dose met (refer to section C.5.2 and to Manual of Operations).

G. SAFETY MONITORING

All adverse events grade 2 or greater will be reviewed in real time by the study investigators, who will establish their relationship to study treatment (see below). The study investigators will also determine whether specific treatment for each adverse event is warranted, whether they meet criteria for study treatment discontinuation, and whether they constitute an emerging exclusionary criterion.

In addition to the real-time review of adverse events, the study data manager and statistician will prepare a bi-yearly report of accrual, early study treatment and study discontinuations (and related reasons), baseline characteristics, longitudinal CD4 T cell count and plasma HIV RNA measurements, Grade ≥2 signs and symptoms, Grade ≥2 laboratory abnormalities and reported serious adverse events (SAEs). This report will be reviewed by the research team and the Safety Monitoring Committee (SMC) during regularly scheduled conference calls every 6 months. The investigators and SMC chair may jointly forgo an actual conference call if the reports have been reviewed and no additional discussion us deemed necessary.

In addition to the regularly scheduled reviews, the SMC will perform expedited reviews of the safety data whenever any of the following happens:

- Two or more participants have experienced a grade 4 AE that is deemed possibly, probably, or definitely related to study treatment
- Any death occurs on study that is deemed possibly, probably, or definitely related to study treatment

Whenever either of the two criteria immediately above occurs, enrollment into the study will be paused until the SMC review has taken place and a determination has been made that enrollment can resume.

The SMC will be composed of an investigator affiliated with Case Western Reserve University in a department other than the Division of Infectious Diseases and HIV Medicine, an investigator not affiliated with Case Western Reserve University, and a biostatistician with no direct responsibilities managing trial-derived data.

H. DATA COLLECTION AND MONITORING AND ADVERSE EVENT REPORTING

H.1. RECORDS TO BE KEPT

CRFs will be provided for each participant. Participants will not be identified by name on any CRFs. Participants will be identified by the participant identification number (PID). Source documents and data will be maintained in accordance with Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/sourcedocpolicy.pdf).

Study personnel will maintain and securely store complete, accurate, and current study records throughout the study. Per US regulations, for each of the investigational products tested, the investigators will retain all study records on site for at least two years after the investigation is discontinued and the US FDA is notified.

Study records will be maintained on site for the entire period of study implementation.

H.2. STUDY COORDINATION

Dr. Benigno Rodriguez will hold the IND for this study.

Study implementation will follow the FDA approved protocol. All amendments will be signed off by the FDA prior to implementation, unless the change is required emergently to assure participant safety.

Study implementation will also be guided by the MOPS instructions and operational guidance on conducting study visits, data and forms processing, specimen collection, processing, and shipping, AE assessment, management and reporting, dispensing study products, documenting product accountability, and other study operations.

Close coordination among protocol team members is necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, retention, follow-up, and AE incidence will be monitored closely by the team.

H.3. ADVERSE EVENT REPORTING

H.3.1. Definitions

H.3.1.1. Adverse event (AE)

An AE is any untoward medical occurrence in a participant who is being administered a study agent/intervention, but does not necessarily have a causal relationship with the study agent/intervention. An AE can therefore be any unfavorable and unintended

sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study agent/intervention, whether or not related to the study agent/intervention.

Adverse events are graded according the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification, August 2009), which can be found on the DAIDS RSC Web Site: http://rsc.tech-res.com/safetyandpharmacovigilance/

H.3.1.2. Adverse Reaction

An *adverse reaction* is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

H.3.1.3. Suspected

A *suspected* adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" indicates that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

H.3.1.4. Unexpected

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the package insert, or is not listed with the same characteristics or severity observed in the trial, or is not consistent with the risk information described elsewhere in the current protocol.

Some adverse events are listed in the package insert as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation.

H.3.1.5. Serious

An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigators or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

Important medical events that may not result in death, are not life-threatening, and do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

H.3.1.6. Life-Threatening

An adverse event or suspected adverse reaction is considered *life-threatening* if, in the view of the investigators, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it hypothetically occurred in a more severe form, might have caused death.

H.3.2. Expedited Adverse Event Reporting

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the investigators or his/her designee must notify the SMC Chair (or qualified alternate) no later than 3 reporting days after the investigators become aware of the event. For the purposes of expedited reporting to the SMC the definition of a "reporting day" in Version 2.0 of the DAIDS EAE Manual will be used, i.e, a reporting day begins at 12:00 AM and ends at 11:59 PM local time, Monday through Friday, holidays falling from Monday through Friday included.

H.3.3. Reporting to the University Hospitals of Cleveland Medical Center (UHCMC) Institutional Review Board (IRB)

In compliance with the UHCMC IRB policy titled "Event Reporting – Unanticipated Problems, Adverse Events, and Protocol Deviations", the Principal Investigators will report all non-fatal events meeting the UHCMC IRB definition of *unanticipated problem involving risk to participants or others* within 14 calendar days of awareness of the event. All fatal events will be reported to the IRB within 7 calendar days of awareness of the event.

H.3.4. Expedited Reporting to the Food and Drug Administration

This study is being conducted under an IND held by Dr. Benigno Rodriguez. Dr. Rodriguez and Dr. Lederman, study co-chairs, will be responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21CFR 312.32).

The Investigators will report in an IND safety report any suspected adverse reaction (see section H.4.1.2) that is both serious (see section H.4.1.5) and unexpected (see section H.4.1.4). If the adverse event does not meet all three of the definitions (serious, unexpected adverse reaction), it will not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after the Investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

H.3.5. Reporting Requirements for this Study

The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study. The study agent for which expedited reporting is required is rIL-2 (PROLEUKIN). In addition to the EAE reporting category identified above, other AEs that will be reported in an expedited manner are:

- All incident cancers
- All opportunistic infections listed in section C.4
- All recurrent serious infections

H.3.6. Grading Severity of Events

Adverse events are graded according the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification, August 2009), which can be found on the DAIDS RSC Web Site: http://rsc.tech-res.com/safetyandpharmacovigilance/

H.3.7. Relationship to Study Agent

The Principal Investigators will determine if there is a reasonable possibility that rIL2 caused or contributed to the AE. The relationship assessment, based on clinical judgment, will rely on the following:

- A temporal relationship between the event and administration of rIL2
- A plausible biologic mechanism for rIL2 to cause the AE
- Another possible etiology for the AE,
- Previous report of similar AEs associated with rIL2
- Recurrence of the AE after re-challenge or resolution after de-challenge.

The terms used to assess the relationship of an event to rIL2 are:

- Definitely related – The AE is **clearly related** to rIL2
- Probably related – The AE is **likely related** to rIL2
- Possibly related – The AE **may be related** to rIL2

- Probably not related – The AE is **not likely related** to rIL2
- Not related – The AE is **clearly NOT related** to rIL2

When an AE is assessed as "not related" to rIL2, an alternative etiology, diagnosis or explanation for the AE will be provided. If new information becomes available, the relationship to assessment of any AE will be reviewed and updated, as required.

H.3.8. Expedited AE Reporting Period

The protocol-defined EAE Reporting Period for this protocol is the entire study duration for an individual participant (from study enrollment until study completion or discontinuation of the participant from study participation for any reason). After the protocol-defined AE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the DAIDS EAE Manual will be reported if the study staff become aware of the events on a passive basis (from publicly available information).

H.3.9. Reporting Procedures

All participants will be followed for possible adverse events throughout their involvement in the study, including issues with study conduct. At each study visit, research staff will elicit participant input as to discomforts or adverse experiences. All grade ≥2 AEs will be reported to the Principal Investigators on a daily basis. A complete blood count, metabolic panel as defined in D.3.5.1, CD4 T cell count, and plasma HIV-1 RNA level will be performed on most visits. The Principal Investigator will obtain these safety data in "real-time" (i.e., within 1-10 days of a study visit) when laboratory values become available. The Principal Investigators will review these data within that timeframe, assess degree of severity, and assess the relationship to study agent/intervention.

The following adverse events will be reported: adverse events graded 3 and above, and any possibly, probably, or definitely related, unexpected, and serious adverse events. These AEs will be reported to the Safety Monitoring Committee (SMC), and FDA. Reporting to the UHCMC IRB will follow current UCHCMC IRB policy (see section H.4.3).

For the purposes of expedited reporting to the SMC the definition of a "reporting day" in Version 2.0 of the DAIDS EAE Manual will be used. Reporting to the UHCMC IRB will follow current UHCMC IRB policy (see section H.3.3).

If at any time, a decision is made to discontinue study agent in all participants, the IND Sponsor (Dr. Benigno Rodriguez), after consultation with the protocol team will inform the US Food and Drug Administration (FDA), the IRB, and the site investigators.

The grading system for drug toxicities is located in the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification, August 2009), available at http://rsc.tech-res.com/safetyandpharmacovigilance.

I. HUMAN SUBJECTS

I.1. IRB Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB of University Hospitals Cleveland Medical Center. A signed consent form will be obtained from the subjects. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, and this fact will be documented in the subject's record.

I.2. Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, NIAID, OHRP, FDA or designee.

I.3. Study Discontinuation

The study may be discontinued at any time by the IRB, the NIAID, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

The SMC will monitor the occurrence of all grade ≥2 signs, symptoms, diagnoses, or laboratory abnormalities.

The SMC will review accrual, toxicity summaries, off-treatment and off-study rates, and reasons broken down by study arms. Meetings of the SMC are described above. The SMC will be asked to consider the following regarding safety events: the grade of the event, the time to resolution (if any), and the site clinician's judgment of the relationship to treatment. The first interim safety review by the SMC is planned 3 months after the first subject enrolls and approximately every 3 months thereafter, but expedited reviews will occur whenever the threshold for temporary enrollment discontinuation is reached. Criteria for temporary enrollment discontinuation and expedited SMC review are:

- Three or more participants have experienced a grade 3 AE that is deemed possibly, probably, or definitely related to study treatment
- Two or more participants have experienced a grade 4 AE that is deemed possibly, probably, or definitely related to study treatment
- Any death occurs on study that is deemed possibly, probably, or definitely related to study treatment

The SMC will review the events whenever any of the criteria above is met, and recommend, based on the results of the review, whether the study can proceed as planned, proceed with modifications, or be discontinued.

I.4. Human Subjects Involvement, Characteristics, and Design

Up to 20 participants will be enrolled in the clinical trial. Potential participants will be recruited primarily from the Special Immunology Unit, the HIV clinic of University Hospitals Cleveland Medical Center, although recruitment from other area hospitals through direct outreach and targeted advertisement is also possible. Characteristics of the study population, inclusion/exclusion criteria, and planned study procedures are described in the clinical protocol. No vulnerable populations will be included in these studies.

I.5. Sources of Materials

Laboratory and clinical staff have received Good Clinical Practices (GCP) and Good Clinical Laboratory Practices (GCLP) training per their role in the study. Clinical monitoring and screening laboratories will be run in CLIA certified labs at University Hospitals Cleveland Medical Center. All specimens will be stored with coded identifiers only in restricted-access laboratories. The CWRU/UHC CFAR Patient Care and Research Database personnel and the study data manager will store, manage and retrieve all study-related data, which will be stored on firewalled servers at UHCMC, in full compliance with relevant HIPPAA requirements. All study records will be stored and accessed using only a participant identifier (PID), and locator data linking identifiable information to the PID will be kept in a separate database in a different location to ensure the confidentiality of these data. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, the FDA, the NIAID, the OHRP or the subject's designees. Information about the subject's participation will not be shared with persons who are not directly involved with the research subjects or as identified in the HIPAA consent.

I.6. Potential Risks and Risk Mitigation Strategies

I.6.1. Risks of rIL-2

Exposure to rIL-2 in this study will be much lower than exposure to rIL-2 when it is used for treatment of malignancy (0.6 million units/kg (42 million units for a 70 kg subject) by intravenous infusion every 8 hrs x 14 doses per cycle) where the greatest toxicities have been observed. In the current study, exposure is minimized by dosing at 5 million units sq twice daily for 5 day cycles every 8 weeks. This starting dose is similar to the starting dose used in the SILCAAT study (4.5 million units twice daily for 5 days every 8 weeks for six cycles) and lower than the starting dose for ESPRIT (7.5 million units sq twice daily for 5 days every 8 weeks for 3 cycles) In those studies, 849 and 2071 patients respectively were randomized to receive IL-2 plus ART. We justify continuing rIL-2 administration for 8 cycles to achieve a similar exposure that was reported in the Chun study that provides rationale for an effect of IL-2 on diminishing HIV reservoir size.

In ESPRIT, there was an excess of grade 4 clinical events in the rIL-2 recipients; the same excess was not seen in SILCAAT. Abrams and colleagues (42) have suggested this is via an impact on procoagulant pathways. In ESPRIT, patients with the highest CD4+ T-cell counts in the rIL-2 arm had the greatest relative risk of clinical events, including death. Perhaps, therefore, receipt of rIL-2 primes the patient for a proinflammatory state subsequently associated with more clinical events, and this is amplified in patients with the greatest CD4+ T-cell response to rIL-2, i.e. the ESPRIT cohort, which had higher baseline CD4+ T-cell counts than in SILCAAT. However, it is still important to remember that while rIL-2 receipt in ESPRIT was associated with more embolic

events, overall there was no excess of opportunistic diseases, death or cardiovascular disease and non-AIDS cancers compared with the ART-only arm.

I.6.1.1. Risk Mitigation Strategy for Serious Infections

Although there was no increased risk of infections in the two large phase III clinical trials of IL-2 administration (SILCAAT and ESPRIT), to mitigate the risk of serious infections, individuals with any of the following conditions will be excluded from enrolling in this study:

- A screening CD4 T-cell count <350 cells/mm^3
- An absolute neutrophil count <1500 cells/mm^3
- An active local or systemic infection
- Use of immunosuppressive or immune modulating therapy
- Use of any investigational agent
- Actively co-infected with HBV or HCV

If a serious infection develops, study drug will be held until the infection is adequately controlled. Study drug administration will be interrupted if the ANC is <1,000 cells/mm^3.

I.6.1.2. Risk Mitigation Strategy for Hypersensitivity Reactions

To mitigate the risk of hypersensitivity reactions including anaphylaxis:

- Patients with a known allergy/sensitivity or any hypersensitivity to components of the study drug or its formulation will be excluded.
- The first dose of study agent will be administered in the Special Immunology Unit that has treated more than 50 patients with IL_2 on SILCAAT and ACTG 328 trials. Subjects will be monitored for a minimum of 30 minutes following the first dose of study drug by personnel with life support training and access to adequate life support equipment and resuscitation medication.
- A severe allergic reaction related to study treatment will be grounds for study treatment discontinuation.

I.6.1.3. Risk Mitigation Strategy for Thromboembolic Disease

In the ESPRIT trial but not among participants in the SILCAAT trial, there was an increased occurrence of vascular events (but not in cardiac disorders) that were seen among 40 of 466 patients receiving IL-2 plus ART and 14 of 386 receiving ART alone. The most frequent vascular event was deep vein thrombosis seen in 10 IL-2 plus ART recipients and in 2 ART alone recipients. To mitigate risk for vascular events in this trial, persons with a history of venous thrombosis will be excluded from participation and at each visit, signs and symptoms of venous thrombosis will be sought.

I.6.1.4. Risk Mitigation Strategy for Laboratory Abnormalities

I.6.1.4.1. Neutropenia

The risk mitigation plan for neutropenia includes:

- Participants with an ANC <1500 cells/mm^3 will not be permitted to enroll in the study;
- ANC will be measured at each clinic visit
- Should the ANC decrease to ≥500 but ≤1,000 cells/mm^3, the rIL-2 dose will be interrupted until the ANC is >1,000 cells/mm^3 and then rIL-2 will be restarted at 3.5 million units twice daily. If the ANC remains above 1,000 cells/mm^3, this dose will be continued until the end of the study.
- Should the ANC decrease ≥500 but ≤1,000 cells/mm^3 while on the reduced dose of 3.5 million units twice daily, rIL2 will be discontinued until the ANC reaches 1,000 cells/mm^3, and then resumed at a dose of 2 million units twice daily. If the ANC then remains above 1,000 cells/mm^3, this dose will be continued until the end of study. If the ANC falls again to ≤1,000 cells/mm^3 while on the reduced 2 million unit twice daily dose, rIL2 will be permanently discontinued.
- Should the ANC decrease to <500 cells/mm^3, rIL_2 will be discontinued.

I.6.1.4.2. Thrombocytopenia

The risk mitigation plan for thrombocytopenia includes:

- Participants with a platelet count <150,000/mm^3 will not be enrolled in the study;
- Platelet counts will be measured at each clinic visit;
- Should the platelet count decrease to ≥50,000 but ≤100,000/mm^3 the rIL-2 dose will be interrupted until the platelet count is >100,000/mm^3 and then IL-2 will be restarted at 3.5 million units twice daily. If the platelet count remains above 100,000 /mm^3, this dose will continue until the end of the study.
- Should the platelet count drop to ≥50,0000 but ≤100,000/mm^3 while on the reduced dose of 3.5 million units twice daily, rIL2 will be discontinued until the platelet count reaches 100,000/mm^3 and then rIL-2 will be restarted at 2 million units twice daily. If the platelet count remains above 100,000/ mm^3 , this dose will be continued until the end of the study. If the platelet count falls again to ≤100,000/mm^3 while on the reduced 2 million units twice daily, rIL2 will be permanently discontinued.
- Should the platelet count decrease to <50,000/mm^3 ,rIL-2 will be discontinued.

I.6.1.4.3. AST and ALT Abnormalities

The risk mitigation plan for AST or ALT abnormalities includes:

- Participants with an AST or ALT $\geq$ 2.5x ULN will be excluded from the study.
- Patients with cirrhosis, severe liver disease or active or chronic hepatitis B or C infection will be excluded from the study.
- AST and ALT will be evaluated at each clinic visit.
- For AST or ALT values > 3x ULN, the rIL-2 will be discontinued. rIL-2 may be resumed at 3.5 million units twice daily when AST or ALT fall to < 2x ULN. If AST and ALT values then remain below 3X ULN, this dose will be continued until the end of the study.
- If AST or ALT values rise again to > 3X ULN while on the reduced dose of 3.5 million units twice daily, rIL-2 will be discontinued until AST and ALT fall to <2X ULN and then resumed at a dose of 2 million units twice daily. If AST and ALT values then remain below 3X ULN, this dose will be continued until the end of the study.
- If ALT values rise again to > 3x ULN while the patient is receiving 2 million units twice daily, rIL-2 will be discontinued permanently.

I.6.1.4.4. Thyroid Abnormalities

The risk mitigation strategy for thyroid abnormalities includes:

- Volunteers with abnormal thyroid function tests or elevated levels of anti-Tpo antibodies at screening will be excluded.
- Participants who develop symptomatic thyroid disease or abnormal T3 or T4 serum levels will be required to discontinue study treatment permanently.
- Participants who develop TSH levels ≥3.99 mIU/L will be required to discontinue study treatment permanently.
- Participants who develop elevated anti-Tpo antibodies will be required to discontinue study treatment permanently.

I.6.1.4.5. Renal Dysfunction

The risk mitigation strategy for renal function abnormalities includes:

- Participants with serum creatinine >1.5 mg/dL will be excluded from the study, and serum creatinine will be evaluated at each clinic visit.
- Should the serum creatinine increase to >2.0 mg/dL but ≤3 mg/dL, rIL-2 will be interrupted. rIL-2 may be resumed at 3.5 million units twice daily when creatinine falls to <1.5 mg/dL. If the creatinine remains ≤1.5 mg/dL on the reduced dose of 3.5 million units twice daily, this dose will be continued until the end of the study.
- If serum creatinine rises again to > 1.5 mg/dL while on the reduced dose of 3.5 million units twice daily, rIL-2 will be interrupted until creatinine falls to

< 1.5 mg/dL and then rIL2 may be resumed at 2 million units twice daily. If serum creatinine then remains below 1.5 mg/dL, this dose will be continued until the end of the study. If serum creatinine rises again to >1.5 mg/dL while on the reduced dose of 2 million units twice daily, rIL2 will be permanently discontinued.

- Should serum creatinine rise to > 3.0 mg/dL , rIL-2 will be discontinued permanently

I.6.1.5. Risk Mitigation Strategy for Malignancies

To minimize the risk or emergent or recurrent malignancies, subjects with a history of or active cancer will be excluded, and a review of interval diagnoses or medical events and a full or targeted physical exam will be performed at each visit. Additionally, concomitant immunosuppressive medications will be prohibited during the study.

I.6.1.6. Risk Mitigation Strategy for Viral Reactivation

The risk mitigation strategy for viral reactivation includes:

- Volunteers with evidence of active hepatitis B or C infection will not be enrolled in the study.
- Participants who develop herpes zoster will be required to discontinue treatment until completion of treatment and resolution of symptoms.
- Participants who develop a recurrence of herpes zoster or murtidermatomal involvement will be required to discontinue study treatment permanently.
- Participants who develop a detectable HBV DNA while on study will require permanent study discontinuation.

I.6.1.7. Risk Mitigation Strategy for Blood Sample Collection and Intavenous Infusion

The most common risks of blood sample collection are pain at the puncture site, bruising, and a feeling of lightheadedness. To minimize these risks, blood draws will be performed by trained personnel, and will be performed in a secure environment with access to first aid equipment, bandages, and trained healthcare professionals.

I.7. Adequacy of Protection against Risks

I.7.1. Recruitment and Informed Consent

Before the initiation of any study procedures, subjects will be informed about the study and asked to sign an IRB approved informed consent/HIPAA document. Subjects will be consented in a private exam room. Subjects will be given time to read the consent, ask questions and consider the risks and/or benefits to participation in this research study prior to obtaining their signature. All subjects enrolled in the study will be given a copy of their signed and dated informed consent document. This consenting process will be done by trained research staff at the Case Clinical Trials Unit.

I.7.2. Protections against Specific Study-related Risks

An extensive risk mitigation strategy has been implemented to address risks derived specifically from the study treatments and procedures. Details of the risk minimization strategy for each risk category are provided above. All study-related activities will be conducted in private areas to protect patient confidentiality.

I.8. Potential Benefits of the Proposed Research to Human Subjects and Others

Participants in this trial are not expected to derive a direct benefit from their participation. If this trial provides proof of concept for the notion that rIL-2 decreases the size of the persistent HIV reservoir in peripheral blood, the results could offer the starting point for the implementation of a therapeutic strategy to cure or induce ART-free remission of HIV infection and that could result in a tangible clinical benefit to persons living with HIV/AIDS, including those directly involved in the study.

I.9. Importance of the Knowledge to Be Gained

As there is great community and scientific interest in developing strategies to allow cure or remission of HIV infection, confirmation of a significant effect of rIL-2 administration on indices of HIV persistence will be important to both enhance understanding of mechanisms of persistence as well as to provide a therapeutic strategy that could contribute to HIV eradication or remission.

I.10. Data and Safety Monitoring Plan

The two PIs, Drs. Rodriguez and Lederman, both experienced HIV clinical trialists, will review all adverse events, determine their relationship to study procedures, and generate reports to the IRB as needed. The data manager will prepare data reports every four months, and inconsistencies in data elements will be resolved by review of primary sources, interview with research personnel, and general consensus among study team members. The protocol statistician will be responsible for preparing the overall data reports, and for alerting the team to trends in adverse event frequency or data discrepancies. Trial safety will be monitored by an SMC.

I.11. ClinicalTrials.gov Requirements

When regulatory approval is secured, this study will be registered with ClinicalTrials.gov. Results information (including adverse events) will be reported no later than 1 year after the primary completion date per Food and Drug Administration Amendments Act (FDAAA) requirements (http://grants.nih.gov/ClinicalTrials_fdaaa/steps.htm).

I.12. Inclusion of Women and Minorities

Both men and women will be included in the trial. Minorities, including Spanish-speaking persons, will be eligible to enroll in the trial, and a Spanish language consent will be available for them.

I.13. Inclusion of Children

Children ages 18 to 21 will be included in this study.

J. PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be decided by consensus of the study team.

K. BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All dangerous goods materials, including diagnostic specimens and infectious substances, will be transported using packaging mandated by CFR 42 Part 72, as per the International Air Transport Association (IATA) Dangerous Goods Regulations.

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