Abbreviated Title: Ph I Study of CIV rhIL-15
CC Protocol #: 12-C-0113 J
Version Date: 06/13/2018
NCT Number: NCT01572493

A PHASE I STUDY OF A CONTINUOUS INTRAVENOUS INFUSION OF RECOMBINANT HUMAN IL-15 (rhIL-15) IN ADULTS WITH METASTATIC CANCERS

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Investigational Agents:

Drug Name:	Recombinant human interleukin-15 (rhIL-15)
IND Number:	100820
Sponsor/IND Holder:	Center for Cancer Research, NCI
Manufacturer:	Biopharmaceutical Development Program, NCI

Commercial Agents: None

PRÉCIS

Background:

- Interleukin-15 (IL-15) is a stimulatory cytokine with a number of desirable immunotherapeutic features, and clinical trials evaluating recombinant human (rh) IL-15 are underway.
- In contrast to IL-2, IL-15 treatment does not stimulate activation-induced cell death of T-cells; potentially inhibits immunosuppressive $CD4^+CD25^+$ T regulatory cells, contributes to the proliferation, differentiation and activation of $CD8^+$ T-cells and NK-cells and the maintenance of long-term $CD8^+$ memory T-cells.
- IL-15 is active in a number of syngeneic mouse preclinical tumor models, and vaccinia-based constructs expressing IL-15 induced long-lasting, high-avidity cytotoxic $CD8^+$ T-lymphocyte response that appears to be more effective than similar IL-2–expressing vaccines.
- Pharmacology/toxicology (pharm/tox) experiments in non-human primate (NHP) rhesus macaques and preliminary results from the first-in-human phase I trial examining rhIL-15 given as an IV bolus (IVB) for 12 consecutive days indicate significant stimulation and expansion of NK-cells and $CD8^+$ T-cells.
- rhIL-15 given as an IVB at 1 mcg/kg dose level appears to be well tolerated despite the presence of some common cytokine-related side effects indicating that 0.1 mcg/kg/day is an appropriate initial dose level for a phase I safety trial of continuous intravenous infusion (CIV) of rhIL-15.
- Comparison of the pharmacokinetic and immunologic assessments from the IVB phase I trial with the data from both sets of NHP pharm/tox experiments suggest that CIV of rhIL-15 may have greater potential for stimulating an anticancer cellular immune response with a more manageable safety profile.

Objective:

- Determine the safety, toxicity profile, dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of rhIL-15 administered as a CIV for 10 consecutive days (240 hours) or for 5 days (120 hours) in subjects with metastatic unresectable cancers for which curative or palliative measures either do not exist or are not associated with a survival advantage.

Eligibility Criteria:

- Patients ≥18 years-old, ECOG PS ≤ 1, with pathologically confirmed metastatic unresectable cancers for which curative or palliative measures either do not exist or are not associated with a survival advantage.
- Patients with measurable or evaluable disease, normal organ and bone marrow function

Design:

- This is a single-institution, open-label, non-randomized 3 + 3 design phase I dose-escalation study.
- Groups of 3 to 6 subjects will receive CIV rhIL-15 at doses of 0.1, 0.25, 0.5, 1, 2, and 4 mcg/kg/day for 10 days provided that DLT has not been observed.

- After assessments of the 10-day dosing cohorts have been completed, new groups of 3 to 6 subjects will receive CIVrhIL-15 at doses of 3, 4 and 5 mcg/kg/day for 5 days provided that a DLT has not been observed.
- Patients with evidence of response and the absence of significant toxicities will be eligible for repeat cycles of treatment.
- Samples for correlative studies will be obtained prior to treatment and at specific times points during and after treatment to assess pharmacokinetics of rhIL-15, the effect of rhIL-15 on immune cell subset populations and pro-inflammatory cytokine levels in the peripheral blood and for the development of neutralizing anti-rhIL-15 antibodies.

SCHEMA

Patient Cohort	Number of Patients	Dose of CIV rhIL-15 (mcg/kg/day x 10 days)*
1	3 to 6	0.1
2	3 to 6	0.25
3	3 to 6	0.5
4	3 to 6	1
5	3 to 6	2
6	3 to 6	4
		Dose of CIV rhIL-15 (mcg/kg/day x 5 days)**
7	3 to 6	3
8	3 to 6	4
9	3 to 6	5

*Nine total patients will be treated at the MTD. The MTD for the 10-day dosing schedule has been identified as 2 mcg/kg/day.

The MTD has not yet been identified for the 5-day dosing schedule.

** If there is a confirmed response (partial or complete response) in at least one patient in the 5-day dosing cohorts, a total of nine patients will be treated at the MTD or the maximum administered dose. Otherwise, there will not be an expansion cohort for any dose in the 5-day dosing cohorts.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Determine the safety, toxicity profile, dose-limiting toxicity (DLT), and maximum tolerated dose (MTD) of recombinant human interleukin-15 (rhIL-15) administered as a continuous intravenous infusion (CIV) for 10 consecutive days (240 hours) or for 5 days (120 hours) in subjects with metastatic unresectable cancers for which curative or palliative measures either do not exist or are not associated with a survival advantage.

1.1.2 Secondary Objectives

- Determine rhIL-15 pharmacokinetics (PK) and the biological effects of rhIL-15 on immune cells reflected in the changes in the percentages and absolute numbers of circulating lymphocytes (T and NK cells) and T cell subsets (naïve, central or effector memory subsets) based on flow cytometric analysis of CD56, CD3, CD4, CD8, CD45RO, CD45RA, CD28, CD95, CCR7 and CD62L expression and on the plasma levels of pro-inflammatory cytokines.
- Evaluate the potential antitumor activity of rhIL-15 by assessing the clinical response rate and time to progression in this patient population.
- Assess the nature of the T-cell infiltration and cytokine and check-point inhibitor gene expression by analysis of pre- and post-treatment biopsies obtained from selected patients with easily accessible tumor deposits.

1.2 BACKGROUND AND RATIONALE

1.2.1 Interleukin-15 (IL-15)

The cytokine that is now called interleukin-15 was initially reported nearly simultaneously by two different research groups, one at the NCI and the other at Immunex Corporation (1, 2). Since this discovery, IL-15 has been and remains one of the key focuses of the Waldmann laboratory's research efforts. While not the exclusive proprietor of all the important publications regarding the biology of IL-15, many subsequent reports from this laboratory have described a number of key biologic features and therapeutic potential of IL-15 (3-9). The clinical application of the laboratory effort that defined IL-15's immunologic role and pathophysiologic contribution to human T-cell lymphotropic virus 1 (HTLV-1)–related diseases and T-cell large granular lymphocyte leukemia (LGL) first led to clinical investigations with the anti-IL-15Rβ chain antibody Mikβ1 (10, 11) that still continue. More recently, the long-awaited first-in-human phase I trial evaluating rhIL-15 as an immunotherapeutic was initiated. This trial is examining the safety and efficacy of rhIL-15 given as an IV bolus to patients with advanced incurable metastatic renal cell carcinoma and melanoma and soon will be followed by additional trials by other clinical cancer researchers.

Interleukin-15 is produced primarily by antigen-presenting cells (APCs) like dendritic cells (DCs) but also by other mononuclear cells following encounters with double-stranded RNA, viral, bacterial and fungal pathogens and also type 1 interferons (IFNs) (12-15). While constitutive IL-15 mRNA production is present in a number of cell types, IL-15 protein expression does not always correlate well with mRNA levels and its secretory pathway is rather unique. IL-15 is bound with high affinity by the IL-15Rα chain, which can be membrane bound

or cleaved to produce a soluble version. IL-15 is normally not secreted as a free cytokine in vivo, but most often is with IL-15Rα on the cell surface of APCs and presented in trans to nearby effector T and NK cells (16, 17). Interleukin-15 has a heterotrimeric receptor (IL-15R) composed of a unique alpha subunit (IL-15Rα chain), a beta subunit that it shares with IL-2 (IL-2R/IL-15Rβ chain) and the common gamma-chain (γc) that is also present on the receptors for cytokines IL-4, IL-7, IL-9 and IL-21 (18-20). While IL-15 and IL-2 also have a number of other similarities as activators of NK cells, $CD4^+$ and $CD8^+$ effector T-cells, there are some important biologic features distinctive to each of these cytokines. In contrast to IL-2, IL-15 does not cause activation-induced cell death (AICD), and seemingly has greater capacity to maintain high-avidity $CD8^+$ memory T-cell responses to invading pathogens by supporting the survival of these T-cells (6, 21-23). In addition to promoting the global $CD8^+$ response (24), IL-15 preferentially expands the most active $CD8^+$, $CD95^+$, $CCR7^-$, $CD28^-$ effector memory cells, and increases their T-cell receptor avidity by inducing higher expression of CD8αβ, which as expected augments these cells' functional capacity (25). Administration of IL-15 has also been shown to upregulate IL-15Rα expression, which allows the effector cells to respond to lower local concentrations of IL-15 that resulted in increased production of secondary stimulatory cytokines such as IFNγ and tumor necrosis factor alpha (TNFα) and increased production of lytic molecules such as perforin (26, 27).

In addition to IL-15's well-described effects on cytotoxic $CD8^+$ T-cells and NK cells, a number of recent publications have demonstrated that IL-15 promotes $CD4^+$ cellular responses more than originally recognized (28-30). Again, the effects are more prevalent in the effector memory subset; but more importantly, IL-15 provides limited stimulation of immunosuppressive $CD4^+$ T regulatory cells (T regs). IL-2 is clearly a potent stimulator of $CD4^+$ $CD25^+$ $FoxP3^+$ T-cells, and some reports have demonstrated that IL-15 can inhibit T regs function (31). As research continued into the relationship of IL-15 and T regs, it became clear that the biology is complex (32-35). The data regarding the effects of IL-15 on T regs is conflicting with some reports demonstrating that IL-15 suppresses T reg function (34, 35). It is clear that IL-15 can stimulate T regs directly or indirectly; the specific milieu and associated cells have important contributions in determining IL-15's impact on the nearby T regs. While IL-15 effects on these immunosuppressive cells are again pleotropic, at a minimum, the data indicate IL-15 has inferior capacity to stimulate T regs compared to IL-2 (31-33).

1.2.1.1 Interleukin-15 as an Immunotherapeutic Agent

In the now more than two decades of clinical investigations into immunotherapeutics for the treatment of human malignancies, many agents have been tested, but IL-2 remains the prototypic agent and a central feature of many effective immunotherapy strategies (36, 37). While IL-2 has advantageous features shown to contribute to the *in vivo* anti-tumor immune response, the less desirable consequences of high-dose IL-2 (HD IL-2) treatment such as capillary leak syndrome, hypotension, and the previously cited roles in AICD and stimulation of immunosuppressive T regs must be recognized. Like IL-2, IL-15 has been shown in many model systems to be a potent stimulator of T and NK cell functions, but IL-15 lacks a number of IL-2's negative characteristics and suggests that IL-15 has greater clinical potential as an immunotherapeutic in oncology (38, 39).

Preclinical experiments indicate that IL-15 is superior to IL-2 in the maintenance of NK cells, NK-T cells, intraepithelial lymphocytes and memory phenotype $CD8^+$ T-cells (1-5). The antitumor effect of IL-2 probably results from its ability to expand lymphocyte

populations *in vivo* and to increase the effector functions of these cells, thereby inhibiting tumor growth. The ongoing cellular therapy effort piloted by the NCI Surgery Branch with tumor infiltrating lymphocytes (TILs) and chimeric antigen receptor T-cells (CARs) (37, 40) and the recently published randomized phase III gp100 peptide vaccine trial (41) have demonstrated clinical efficacy for combination treatments with HD IL-2. Although numerous individual reports have listed positive clinical results from less intensive IL-2 regimens, published results from the limited number of comparative trials indicate the HD three times daily (tid) dosing regimen is the most consistently active IL-2 treatment (42, 43). In composite, the current data suggest higher doses of IL-2 must be maintained at a cost of increased toxicities, to be effective. Unlike the standard practice with IL-2, most new rh cytokines being examined in clinical trials are not given as dose-intense inpatient regimens (44, 45). Biologically, we now understand better the limits of stimulation of HD IL-2 on effector T-cells (AICD, T regs) (12, 34, 38) and NK cells as well as the ability of patients to tolerate prolonged IL-2 treatment (36). The current development plan for IL-15 given at an active but not intensive dose coincides with the philosophic shift to less toxic regimens for new immunotherapeutics being evaluated in clinical trials (44-46). Analogous to the investigation into IL-15, a number of new immunotherapeutic agents identified by our increased understanding of a productive immune response are directed at homeostatic or counter-regulatory cellular processes like CTLA4, PD1, indoleamine 2,3-dioxygenase and immunosuppressive T regs or macrophage-derived suppressor cells (MDSC) that are induced sometimes indirectly by IL-2 treatment (33, 46-51). IL-15's considerable ability to activate crucial effector T-cells and NK-cells, its inhibition of AICD, the lack of a meaningful contribution to the generation of T regs, potential for cyclic restimulation of effector cells and its unique capacity to maintain the function of $CD8^+$ memory T-cells; indicate that IL-15 has greater potential than IL-2 as a cancer immunotherapeutic.

1.2.1.2 Interleukin-15 in Preclinical Immunotherapy models

Attempts to prevent tumor growth in mice by administration of IL-15 have proven effective (7, 14, 52). The importance of IL-15 in the elimination of syngeneic MC38 colon carcinoma has been examined using IL-15 transgenic mice that produce large quantities of IL-15. Wild-type C57Bl/6 mice died due to substantial pulmonary involvement by 40 days after infusion of the MC38 carcinoma cells, but similarly exposed IL-15 transgenic mice did not develop such metastases and survived. The role of IL-15Rα-mediated transpresentation in the therapeutic action of IL-15 was evaluated in a related model when the IL-15Rα expression element was transfected into MC38 colon carcinoma cells. The administration of unmodified MC38 cells to wild-type mice led to death within 40 days, whereas mice with normal IL-15 production given IL-15Rα–transfected tumor cells did not develop tumors (53). In this system, IL-15Rα on the surface of tumor cells bound circulating IL-15 present in the blood that was subsequently transpresented to circulating NK-cells, leading to the destruction of the tumor. In analogous non-transfected model, our group demonstrated that intraperitoneal therapy with IL-15 prolonged the survival of mice challenged with the syngeneic CT-26 colon carcinoma cells. Klebanoff and coworkers (7) also showed that IL-15 enhanced the *in vivo* activity of tumor reactive $CD8^+$ T-cells in the T-cell receptor transgenic mouse (Pmel-1) whose $CD8^+$ T-cells recognize an epitope derived from the melanoma antigen gp100 and stimulated the *in vivo* antitumor activity of these T-cells in a B16 melanoma model. The synergy of IL-21 and IL-15 in stimulating $CD8^+$ T-cell expansion and functional capacity also produced apparent cures of established large B16

melanomas (52). In addition, the IL-15 monotherapy control arm in these studies demonstrated significant antitumor effect over untreated controls. Systemic IL-15 given in combination with a fowlpox TRICOM vaccine encoding B7-1, ICAM-1, and LFA-3 improved survival in a mouse subcapsular renal cell cancer model compared to TRICOM vaccination alone or TRICOM plus systemic IL-2 (54).

IL-15 has also been shown to promote anti-viral T-cell responses. Vaccinia vectors co-expressing IL-15 and an HIV antigen glycoprotein 160 (gp 160) induced long-lasting robust cytotoxic T-lymphocyte (CTL) response in contrast to the short-lived T-cell immunity from a vector expressing IL-2. Administration of DNA vaccines containing an optimized plasmid encoding IL-15 improved the function and longevity of $CD8^+$ T-cell response to influenza virus hemagglutinin protein (24). IL-15 was shown to increase long-lived antigen specific memory $CD4^+$ and $CD8^+$ T-cells in rhesus macaques (29, 55-57), and to increase effector memory $CD8^+$ T-cells and NK-cells in Simian immunodeficient virus (SIV)–infected macaques (29). In this study, recombinant rhesus macaque (rm) IL-15 treatment in 9 SIVmac 251–infected cynomolgus monkeys was given twice weekly for 4 weeks at a low dose of 10 mcg/kg and a high dose 100 mcg/kg. Compared to saline controls, rmIL-15 treatment induced a nearly three-fold increase in peripheral $CD8^+CD3^-$ NK cells. Furthermore, $CD8^+$ T-cell numbers increased more than two-fold mainly due to an increase in $CD45RA^-$, $CD62L^-$, and $CD45RA^+$ $CD62L^-$ effector memory $CD8^+$ T-cells.

To support this proposal, the Lymphoid Malignancies Branch has recently performed additional murine experiments to assess the potential of CIV IL-15 (Zhang and Waldmann unpublished) using an analogous subcutaneous (SC) minipump system. Protocol mice were infused intraperitoneally (IP) with MC-38 tumor cells and IP injection or SC minipump treatment of the 5 experimental groups were initiated 1 day later. The 5 treatment groups included a phosphate buffered saline (PB5) control, human IL-15 at 5 mcg/day IP for 14 days (5 x 14), human IL-15 at 2.5 mcg/day IP for 14 days (2.5 x 14), human IL-15 at 5 mcg given every other day IP over 14 days for 7 doses (5 x 7) and human IL-15 via an implanted pump (2.5 mcg/day) for 14 days that demonstrated more favorable pharmacokinetics for 2.5 mcg/day infusion pump route of administration with sustained serum IL-15 levels in 3500 to 8000 pg/mL range in comparison for short high exposures (> 30, 000 pg/mL) for the daily IP injections.

Increases in absolute lymphocyte count (ALC) were seen in the groups receiving rhIL-15 and nearly identical absolute numbers of $CD8^+$ lymphocytes were seen in animals receiving 2.5 mcg/day via the pump as those receiving 5 mcg/day as an IP injection (**Figure 1**, top figure). All treatment regimens produced similar increases in the percentage of NK cells, but increases in the percentage of $CD8^+$ and $CD44^{hi}$ $CD8^+$ terminal effector cells were highest in the animals treated via the pump at 2.5 mcg/day. It is also important to note that IL-15 treatment stimulated the vast majority of the $CD8^+$ cells to become $CD44^{hi}$ activated effector cells. These data suggest that continuous administration of IL-15 as opposed to bolus dosing stimulates different populations of lymphocytes and may be a better dosing schedule to stimulate a T-cell or $CD8^+$ cells response that also includes a slower decline $CD8^+$ cell numbers after the cytokine treatment has been stopped compared to animals treated with a daily IP injection.

1.2.1.3 Results from Non-Human Primate (NHP) Toxicology Studies

The Lymphoid Malignancies Branch had previously performed a toxicology study in NHP

in support of the IND application for the ongoing IVB clinical protocol (56, 57). In this toxicology study, 24 adult rhesus macaques were divided into 4 treatment groups of 6 animals each. The groups of monkeys received vehicle diluent control alone, or rhIL-15 at 10, 20 or 50 mcg/kg/day IVB daily for 12 days. The animals were assessed regularly with clinical observation, physical examination and laboratory tests that included hematology, serum chemistries, coagulation studies, PK analysis of serum rhIL-15, immunologic parameters, a survey of autoimmune markers and a complete necropsy with gross and histopathological examination. After 8 days of treatment, the animal receiving 200 mcg/kg/day and to a lesser degree those animals receiving 50 mcg/kg/day exhibited lethargy, a disheveled appearance, diarrhea and dehydration that in some cases required IV fluids. Laboratory results from this time period showed transient grade 3/4 granulocytopenia in the rhesus macaques receiving rhIL-15 at a dose of 50 mcg/kg/day, but no infections were seen and concurrent bone marrow samples (aspirate and biopsy) showed a hypercellular marrow containing all elements of the granulocytes series. Histologic examination of the macaques' livers demonstrated granulocytes and other leukocytes in the sinusoids adjacent to normal appearing hepatocytes suggesting that redistribution to the tissues as a contributing factor in the apparent neutropenia. Normal or elevated granulocyte counts were restored within 48-72 hours of discontinuation of the rhIL-15 dosing. No antibodies directed against rhIL-15 were detected in the animals, and no animals developed anti-nuclear antibodies (ANA). At necropsy, the only gross or microscopic abnormalities were loss of adipocytes in the hyperplastic marrows and the presence of neutrophils and other white blood cells (WBCs) in the liver sinusoids. No animal deaths occurred on this study.

To support a NIAID-planned sponsored clinical trial, a similar toxicology study was performed to evaluate the effects of CIV treatment and more frequent administration of SC rhIL-15 to rhesus macaques. Fifteen male macaques were divided into 5 groups of animals and were treated with either CIV or SC rhIL-15 as shown below in **Table 1** with one group of animals receiving vehicle alone serving as the control. The SC rhIL-15 was administered either on a twice weekly schedule for 2 weeks or for 10 consecutive days, and the CIV dosing was also given continuously for 10 days. In the initial phase of the study animals receiving CIV IL-15 were subdivided into two groups (groups 2 and 3) to evaluate responses to different preparations of rhIL-15 distinguished by relative levels of deamidation (identified as high-D and low-D forms). Initially the plan was for two treatment cycles separated by an 8-week interval, but the protocol was modified at a later time point to add a third cycle of CIV low-D IL-15 for treatment groups 2 and 3. This third cycle was given approximately 9 months after completion of the second cycle of 10-day continuous infusion IL-15. The test animals were assessed for clinical toxicity, had standard biochemical and hematologic laboratory tests and several immunologic parameters similar to the previous IVB NHP study. The tests also included monitoring serum IL-15 PK, production of secondary cytokines in animals receiving CIV rhIL-15, development of antibodies to IL-15, and changes in lymphocyte subsets by flow cytometry.

Table 1: Treatment Groups Rhesus Macaques Toxicology Experiment

Group	Treatment	Dose Level in mcg/kg/day	Route of Administration	Duration of Treatment	Total Doses/ Cycle	# Cycles
1	Vehicle Control	0	Continuous Infusion	10 Days Consecutive	10	2

Group	Treatment	Dose Level in mcg/kg/day	Route of Administration	Duration of Treatment	Total Doses/ Cycle	# Cycles
2	High-D IL-15	20	Continuous Infusion	10 Days Consecutive	10	3
3	Low-D IL-15	20	Continuous Infusion	10 Days Consecutive	10	3
4	Low-D IL-15	20	Subcutaneous	2x per week 2 weeks	4	2
5	Low-D IL-15	20	Subcutaneous	10 Days Consecutive	10	2
6	Low-D IL-15	40	Subcutaneous	10 Days Consecutive	10	2

While no consistent change in the initial phase test animals' body weights was observed during the first 10-day treatment, a number of animals in both CIV arms had decreased food consumption, diarrhea, rough hair coat, languid posture most notable from day 7 onward but appearing to resolve completely by day 13 or 14. These findings correlated with changes in the clinical chemistries that yielded treatment-related abnormal results including elevated mean serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), decreased albumin, total protein and calcium concentrations in animals given either 20 mcg/kg high-D or low-D CIV in the day 7 and 10 routine laboratory assessments. The changes in the ALT and AST values were not sufficiently abnormal to be considered significant at day 7, but had progressed by day 10 and were considered a significant adverse treatment effect in these test animals at this time point. The changes observed for albumin, total protein and calcium were considered moderate and to be another reflection of the IL-15 treatment effects on the liver. Diverging somewhat with the clinical observations, the adverse treatment-related increases in ALT persisted to day 17 in animals given CIV rhIL-15. The transaminase elevations had returned back to normal values by day 24 laboratory assessments and remained in the normal range when repeated on days 40, 46, 53, 60 and 67 during treatment cycle 2. However, within 1 day of beginning the second course of high-D CIV treatment, one test animal was discovered dead on the second morning of the course. The animal had not exhibited any signs of significant toxicities either by inspection or laboratory analysis on day 1 and actually had been gaining weight over the course of the study. Microscopic examination showed mild to severe autolysis of tissue from many organs resulting in loss of cellular detail that did not allow full evaluation of certain organs. There were submucosal hemorrhages seen in all parts of the small bowel that was most severe in the cecum, but there was an absence of inflammatory cells in all the areas examined microscopically. Moderate vascular congestion was seen in several organs and mild alveolar edema was noted in the lungs, but both of these findings were felt to be most likely related to terminal events. Because of the findings in the small bowel, intussusception was considered as a possible cause of this animal's death, but this was not seen at the initial examination of the animal's abdominal cavity. Ultimately, no clear cause of death was identified in this animal based on the gross and microscopic results.

There was no consistent difference noted in the immune activation or lymphocytosis produced by the high and low D rhIL-15 preparations. Compared to the lymphocytosis noted in the NHP IVB toxicology study, the increase in WBCs and ALC noted in the groups (high D and low D combined in graphs) receiving the 20 mcg/kg CIV dose represented is a striking (**Figure 1**) with ALC showing 20-fold expansion from baseline by the day 10 time

point and accounting for approximately 90% of leukocytes. This lymphocytosis is still substantially present through day 17, a week after the IL-15 treatment has been stopped and it is not until day 24 that the primates WBC and ALC have returned near to their baseline levels. FACS results assessing changes in lymphocyte subsets shown below in **Figure 2** demonstrate modest increases in $CD4^+$ cells, substantial increases in ($CD3^-$ $CD16^+$) NK cells, but dramatic increases in $CD8^+$ lymphocytes that account for the majority of the increases in ALC. The increases in $CD4^+$ lymphocytes were primarily in the central memory ($CD28^+CD95^+$) subset whereas the increases in $CD8^+$ cells were due to modest increases in the central memory subset and 20- to 100-fold increases in the effector memory ($CD95^{\pm}$ $CD28^-$) subset (data not shown) paralleling the results observed in the recently performed murine IP experiments.

Figure 1: **White Blood Cell Count and Absolute Lymphocyte Count**

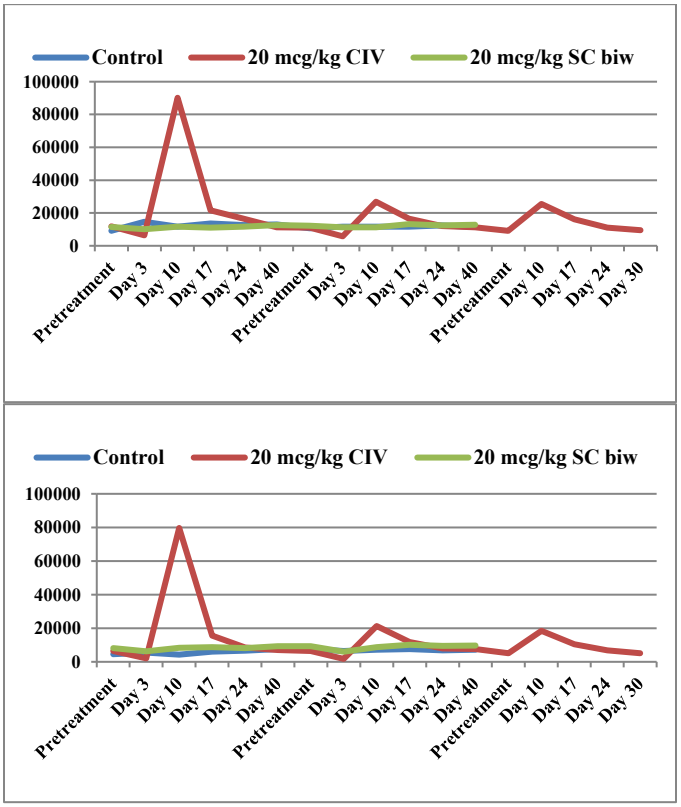
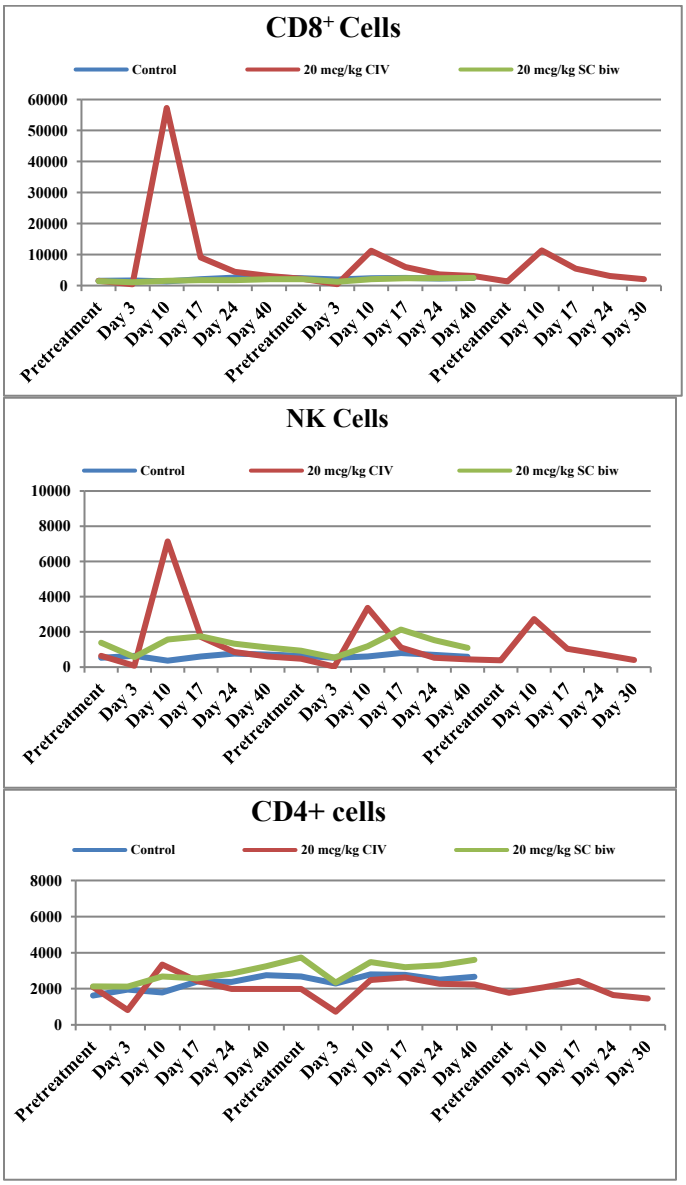


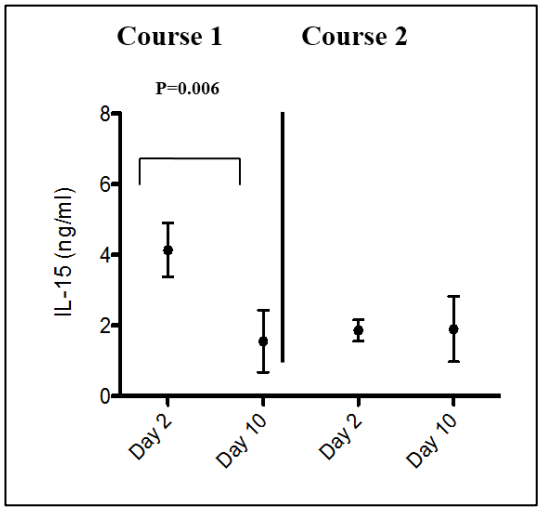
Figure 2: Absolute Number of $CD8^+$, $CD4^+$ and NK Cells



Extensive PK was not performed in the animals receiving CIV rhIL-15, with only single time points on days 2 and 10 of infusion assessed. IL-15 levels reached the low ng/mL level on day 2 and ranged from 2.1 to approximately 6.3 ng/mL. Day 10 values during course 1 were generally lower. During subsequent courses the peak values observed were also in the ng/mL range, and in some animals, the day 10 values were higher than on day 2 (**Figure 3**). Day 17 and 24 samples (7 and 14 days after last day of infusion) had no detectable human IL-15 in the serum. The greater than 20-fold expansion of IL-15–responsive cells (presumably IL-15 receptor–positive cells) might contribute to the decrease in free circulating rhIL-15 measured at the day 10 time point, but the differences in IL-15 PK during course 2 as well as decreased course 2 and 3 leukocytosis are not yet explained and remain under investigation. Preclinical testing of rh cytokines in NHP provides valuable preclinical information for clinical trials; species differences will occasionally cause confounding results, but these issues do not diminish the provocative positive results from

these experiments.

Figure 3: IL-15 Levels



An important cause of the PK and stimulation differences to be eliminated was the development of rhesus macaque (rm) antibodies against human IL-15. The rhesus monkeys were evaluated for the presence of rm anti-IL-15 antibodies, and in the monkeys receiving CIV IL-15, 3 of 6 animals developed detectable levels of antibodies at some point during the test period. One animal became positive after the first course with a boost in the IL-15 titer in this animal positive after the second course. Another CIV animal became low titer positive after the second course and remained positive in the pre-course 3 assessment, and a third animal was observed to have antibodies to IL-15 that were just above the lower limit of detection of the assay in the pre-course 3 assessment. All 3 animals continued to have elevated titers after completing the third course of CIV IL-15. While the test animal that demonstrated anti-IL-15 antibodies after the first course of treatment and had the highest anti-cytokine titers throughout the experiments had somewhat lower WBC and ALC values measured for the first treatment course, this particular macaque and none of the other antibody-positive animals showed substantial differences in their lymphocyte expansion or pattern of lymphocyte subpopulations compared to animals that tested negative for anti-IL-15 antibodies. The underlying causes of the difference in the immune response observed in courses 2 and 3 treatment has not been fully explained and is still being assessed with samples obtained during these experiments.

The macaques also had an inflammatory cytokine analysis performed. At the time points measured in this study, IL-8 was the only cytokine that gave values that were within the detection range of the standard curve, while all other samples had signals below the 2.4 pg/mL test standard. It is possible that evaluation of secondary cytokine induction would be more successful if the focus was on early time points such as 4, 8 and 24 hours after initiation of continuous infusion that have been measured in the ongoing IVB trial and are planned for this trial.

Compared to NHP that had received 20 mcg/kg/daily as an IVB for 12 days, the animals receiving 20 mcg/kg/daily CIV for 10 days had more constitutional and gastrointestinal side effects, as well as biochemical laboratory abnormalities that were most noticeable after the

first week of course 1 treatment coinciding with the period of greatest lymphocytosis. These side effects, biochemistry abnormalities and lymphocytosis, were somewhat blunted during the second and third treatment course for reasons that are again not yet entirely clear.

1.2.1.4 Clinical Experience from the Completed IV Bolus Phase I Study

The first in-human (FIH) trial that tested a daily 30-minute infusion (IVB) for 12 days in subjects with metastatic melanoma and renal cell cancer has been completed after treating 18 subjects (58). Doses assessed in this trial were 3, 1 and 0.3 µg/kg; with the 0.3 µg/kg dose determined to be the MTD for this schedule. DLTs at the 3 µg/kg dose were grade 3 hypotension and grade 3 thrombocytopenia; DLTs at the 1 µg/kg dose were grade 3 elevations of transaminases. One Serious Adverse Event (SAE) grade 3 pneumonitis possibly related to rhIL-15 treatment occurred during a subject's second cycle of treatment. The frequency and severity of common cytokine related toxicities such as fever, rigors, capillary leak syndrome and edema were generally proportional to the dose of rhIL-15. Abnormal laboratory results were seen in all subjects and most often were CTC grade 1 or 2 events. Laboratory adverse events (AEs) were less clearly proportional to the dose of rhIL-15 both in frequency and severity as indicated by low and transient occurrence of grade 2 laboratory AEs in the 3 µg/kg cohort, whereas both DLTs in the 1 µg/kg dosing cohort were elevated AST/ALT. The 0.3 µg/kg dosing cohort also had a high frequency of laboratory abnormalities including non-DLT grade 3 elevation of ALT/AST in one patient. There was a consistent temporal pattern of post-treatment AEs most clearly seen in 3 µg/kg cohort with fever beginning approximately 2 to 2½ hours after the rhIL-15 infusions, then rigors followed almost immediately by decreased blood pressure (BP).

A similar but less severe set of post treatment toxicities were seen in the 1 µg/kg subjects with chills rather than rigors, less significant increases in temperature, but no appreciable BP changes. There was a very low frequency or absence of these post infusional events in the 0.3 µg/kg dosing cohort. Serial analyses of inflammatory cytokine levels (IL-1β, IL-6, IL-8, IL-10 and TNFα) showed marked increases in serum levels of these inflammatory cytokines that coincided with these clinical toxicities and was most closely matched with the IL-6 levels. As predicted by the NHP toxicology studies performed to support this trial appreciable expansion and activation was seen for T-cells and NK cells, but the increase in lymphocytes was statistically significant only for the group that received the 3 µg/kg dose that produced a 10-fold increase in NK cells, a more than 3-fold increase in T-cells mostly due to expansion of the terminally differentiated (memory and effector memory) $CD8^+$ cells. The half-life (t ½) was approximately 2 1/2 hours with Cmax 47,800, 15,900 and 1260 pg/mL respectively for the 3 dose levels tested. There were no clinical responses, with stable disease as a best response. However, 5 subjects manifested a decrease between 10 and 30% in their marker lesions, with 2 of these subjects having improvement or clearance of some lung lesions.

1.2.1.5 Previous Clinical Experience for Patients treated in this Protocol

Twenty-five patients have been enrolled and treated in this protocol. Patients 1-10 were treated at the first 3 dose levels (0.1 mcg/kg, 0.25 mcg/kg and 0.5 mcg/kg) and had no DLTs or significant toxicities.

Patients # 11 through 16 were treated at the 1 mcg/kg dose level without additional issues except patient #11 who was taken off treatment because he experienced DLT: grade 3

hypoxia on Cycle 1 Day 6. The patient's respiratory status was improving daily in the 8 days he was observed after this event, but it was not completely resolved by the time he was discharged home. The research team continued to be in contact with the patient after discharge. The patient reported some dyspnea on exertion during the trip from the airport, but was comfortable on the same low flow oxygen when finally settled at home and had no new complaints. When next contacted, the patient's family reported that when the patient was checked approximately one hour after going to bed, he was found to be unresponsive. Paramedics were called and determined that the patient had already expired. No resuscitation attempts were made and a post mortem exam was not performed due to the family's wishes. This death was reported on NIH Problem Form iRIS # 335749.

Patients #17 through 19 tolerated the 2 mcg/kg dose level without significant toxicities or DLT.

Patients #20 and 21 treated at the 4 mcg/kg dose level both had serious DLTs that required early discontinuation of their treatment. Patient # 20 was a 64-year-old male with a history of relapsed refractory high mitotic rate spindle cell gastrointestinal stromal tumor (GIST) of the small bowel which was initially diagnosed in August 2010. The patient began CIV rhIL-15 treatment on 3 February 2015 and initially tolerated his treatment with the expected side effects; but by treatment day 4 he had become very fatigued, agitated and had an inverted sleep cycle. In addition, he had significant increases in his LFTs especially his bilirubin that required interruption of his treatment by day 5. The patient also became hypoxic later that day and required oxygen by nasal cannula to maintain adequate oxygen levels. Interruption of his treatment and attempts to diurese the patient did not improve his symptoms, hypoxia or his laboratory abnormalities and when the patient became frankly encephalopathic on cycle 1 day 6, his treatment was permanently discontinued. A CT pulmonary angiogram confirmed the patient had no pulmonary emboli and over the course of the next few days his abnormal LFTs, encephalopathy, hypoxia and general condition improved to the point that the patient was discharged home. His overall improvement continued for the next 10 to 14 days and he had returned to his pretreatment functional status when he began to complain of increased abdominal/RUQ pain, decreased appetite and worsening performance status. Restaging CT scans performed on cycle 1 day 28 confirmed physical exam findings of worsening hepatomegaly and dramatic progression of his intrahepatic as well as an increase in intra-abdominal disease compared to his pretreatment scans. He was taken off protocol at this time. The patient was assessed as recovered from his acute rhIL-15 toxicities and his late general decline was attributed to massive progression of his hepatic and abdominal disease. The patient was unable to qualify for any additional research studies due to his poor performance status and worsening organ dysfunction. He died in Home Hospice on 28 April 2015.

Patient #21 was a 61year old female with squamous cell carcinoma (SCC) at the base of the tongue (BOT) initially diagnosed in August of 2012 and treated with aggressive combined modality treatment for a complete remission. The patient was discovered to have inoperable locally recurrent or metastatic disease in December of 2014. This patient began treatment rhIL-15 (4 mcg/kg/day by CIV for 240 hours) on 9 February 2015. Her treatment was complicated by significant diarrhea initially considered osmotic due to a change in her G-tube nutritional supplement and later antibiotic related enteritis from empiric treatment with Amoxicillin/Sulbactam (Unasyn®) that eventually required interruption of her CIV rhIL-15

treatment on day 3. The patient's diarrhea improved with a 24-hour interruption of her CIV rhIL-15, but within a few hours of restarting rhIL-15 treatment the patient developed signs of an acute abdomen. She underwent a laparotomy that evening and was found to have patchy ischemia involving all of her stomach, small and large bowel that could not be addressed by surgical resection. A detailed description of her course and her grade 5 Visceral Arterial Ischemia was reported to the IRB in an NIH Problem Form.

The 4 mcg/kg dose level was clearly above the MTD for these 2 patients. As a result, we dropped to the next lower dose level of 2mg/kg for subsequent patients.

Patient # 22 tolerated his 2 cycles of treatment at the 2 mcg/kg dose level relatively well with intermittent temperature spikes concluded to be due to his disease after multiple negative blood cultures and negative UA. This patient did however have prolonged fatigue after both cycles which gradually improved to baseline or near baseline Performance Score (PS) level by his day 28 visits. Post cycle 1 restaging demonstrated stable disease, but his post cycle 2 restaging demonstrated disease progression and the patient was taken off study.

Patient # 23 had metastatic recurrent uterine mixed papillary serous clear cell carcinoma and tolerated both cycles of treatment at the 2 mcg/kg dose level fairly well with the expected side effects of fatigue, fevers, chills but had appreciable rises in her serum creatinine during both cycles of treatment. She also developed progressive dyspnea associated with a moderate right sided pleural effusion and mild hypoxia near the end cycle 1 treatment that required supplemental oxygen by nasal cannula for a brief period. The patient had a therapeutic thoracentesis the day after completing her 240 hours of CIV rhIL-15 after which her dyspnea and hypoxia resolved. The patient had returned to her baseline PS within a few days of completing her cycle 1 and after her restaging scans documented stable disease, she went on to receive her second cycle of treatment. She tolerated her second cycle of treatment with less dyspnea, developed a smaller pleural effusion and only a relative decrease in her oxygen saturation that did not require supplemental oxygen, but again had a therapeutic thoracentesis after completing treatment to improve her respiratory status. Analyses of both thoracenteses showed exudative pleural fluid with ≈ 400 to 650 WBCs/mm3 primarily lymphocytes, few neutrophils, some "other cells" possibly tumor cells and were microbiologically sterile. The patient was known to have fairly extensive right sided sub-diaphragmatic metastatic disease and had previously undergone debulking surgery that included resection of some of her right hemi-diaphragm. We concluded that these treatment-related effusions represented rhIL-15 related inflammation at this site of tumor because the patient did not develop a left sided pleural or any other body cavity effusion during her treatment. The patient's post cycle 2 restaging showed stable disease; she was taken off treatment and remains on study without any treatment related issues.

Patient # 24 who had a diagnosis of NSCLC with EGFR mutation (exome 19 deletion normally associated with EGFR-TKI responsiveness) received one cycle of therapy at the 2 mcg/kg dose level. The patient was noted to have a new right inferior temporal lesion on her cycle 1 day 28 CT scan of the neck. She had an MRI of the brain which showed at least 7 new enhancing lesions that were consistent with new CNS metastases. The patient was started on dexamethasone and sent for consultation with Radiation Oncology. She was taken off study for disease progression and initiation of another salvage therapy.

Patient #25 tolerated 2 cycles of treatment without any significant toxicities. Treatment was

discontinued after the post cycle 2 restaging which demonstrated no significant improvement in her disease and she was subsequently taken off protocol when restaging scans performed at home showed disease progression.

The protocol and the IND were placed on clinical hold in May 2015 due to concerns about pharmacy infusion preparation procedures after discovery of several vials of human serum albumin contaminated with fungus in the lot that had been used to prepare infusion mixtures for 4 of the patients treated in this protocol (NIH Problem Form iRIS # 346817). No evidence of infection or unusual clinical events was seen in a period of prolonged observation and the FDA has recently specified changes in the drug preparation procedure that will allow the protocol to resume.

Since resumption of the protocol in 2017, the last two patients #26 and 27 in the 2 mcg/kg expansion cohort for the 10-day regimen have completed their treatment without unexpected problems. The first two patients #28 and 29 treated with 3 mcg/kg on the 5-day regimen have completed at least one cycle of treatment without unexpected problems or DLT.

1.2.1.6 Rationale for Phase I Evaluation of Repeated Cycles of rhIL-15 given on either a 10-day or 5-day CIV Schedule

When rh cytokines first became widely available for clinical trials, in addition to identifying appropriate dosing, different routes of administration (IVB, CIV, SC, intracavitary or IP) and schedules were evaluated. At the time the NCI Surgery Branch was piloting the three times daily (tid) IVB regimen that has become the standard HD IL-2 regimen, the so called "West" regimen with CIV HD IL-2 given over 5 days (120 hours) was being investigated in numerous clinical trials. The initial publication for this CIV regimen reported a higher response rate (RR) along with a similar spectrum of typical cytokine-induced side effects, but did not show the roller coaster (peak valley) course of BP, capillary leak and widened A-a gradient characteristic of the tid HD IL-2 regimen (59, 60). An important feature that made this treatment plan popular with certain practitioners was the gradual emergence of more serious or DLTs as the patient's limit of tolerance was approached more slowly. The every 8 hour IVB HD IL-2 regimen can be characterized by acute onset of respiratory and vascular toxicities with significant variation depending on the active drug levels related to the most recent dose; these toxicities can occur early in the treatment cycle. In addition, patients can rapidly transition from a tolerable treatment course to severe complications in the space of a few consecutive doses (36). With CIV HD IL-2, there is no rapid variation in immune activation or secondary cytokine levels, and the patients approach their unacceptable toxicity threshold at a steadier and usually slower rate. This CIV HD IL-2 regimen has not been popular for quite some time, but lower-dose CIV regimens have been evaluated by the French Immunotherapy Group (60), in the treatment of patients with HIV (61). It is also interesting to note that recently a positive phase III licensing trial for the anti-GD2 chimeric antibody ch14.18 in the treatment of advanced pediatric neuroblastoma (62) used a CIV IL-2 regimen as part of a multi-agent treatment. This trial was initiated with a 6-week cycle length based on data from the NHP toxicology study that validated the treatment regimen given every 6 weeks. An ongoing clinical multicenter trial (NCI protocol 13-C-0045 or CITN 11-02) evaluating a similar rhIL-15 SC outpatient regimen (M-F for 2 consecutive weeks) has demonstrated that rhIL-15 can safely be given on a 4-week schedule. Patients need to demonstrate some evidence of a clinical response during their first 2 cycles of treatment to be eligible for additional treatment cycles. The clinical course

of patients treated with repeated treatment cycles 4 weeks in length restaged every other cycle allowed by previous amendments has not demonstrated any new or unexpected problems.

The safety data from the NHP toxicology study demonstrated increased systemic, GI, constitutional and biochemistry side effects in the test animals that appeared to coincide with lymphocytosis arising near the end of the first week of treatment. In this proposed study to maximize patient safety, all patients will be treated as inpatients, have frequent clinical (blood pressure, heart rate, respiratory status including pulse oximetry) monitoring and at least daily laboratory assessments. The current rhIL-15 clinical experience has provided good fundamental understanding of the biologic effects of this cytokine, the limitations of preclinical models and NHP pharm-tox experiments to predict all events in humans is clearly evident. Current results strongly indicate that transient biochemical abnormalities such as elevated liver function tests, decreased leucocytes, lymphocytes and platelets can normalize even if CIV rhIL-15 is continued and will resolve to baseline after discontinuation of treatment. Unlike the patients treated with the higher doses in the IVB trial, none of the CIVrhIL-15 treated patients through the 2 mcg/kg dose level have demonstrated meaningful blood pressure changes, spiking fevers or rigors noted in the rhIL-15 IVB patients. There has been no correlation between IL-15–induced lymphocytosis and the intensity of side effects for patients treated in this study or with the IVB regimen that indicate CIV rhIL-15 reproduced the striking lymphocytosis seen in the NHP toxicology studies would automatically be at higher risk for SAEs. Hematologic and immunologic data from patients previously treated in this trial and the SC trial (protocol 13-C-0045) (63-65) also have indicated some divergence in the rhIL-15 effects in humans compared to NHP that should be investigated. In contrast to the NHP data, the CIV and SC rhIL-15 treated patients are showing appreciably greater in vivo increases in absolute NK cell numbers (20-50x pretreatment), but much lower increases in CD8 T-cells (≈ 2-3x pretreatment) albeit at lower rhIL-15 doses than was well tolerated in the NHP. There is preliminary data suggesting differences in the kinetics for upregulation of the IL-15 receptor β chain (CD122) between NK and CD8 cells for the patients treated in this trial that may play a role in this finding. The safety profile and AEs for the patients treated at the 0.5, 1 and 2 mcg/kg dose levels were not appreciably different and suggested the true MTD for the CIV regimen was higher. The SAEs that occurred for the 2 patients treated at the 4 mcg/kg dose level were totally unacceptable and by conventional assessments indicated the MTD had been exceeded for the CIV regimen, but there are unique features to each patient that should be recognized. The start of patient #20's treatment was delayed 6 weeks for approval of a prior amendment during which time his disease became abruptly more aggressive resulting in substantial progression of his hepatic disease burden. The patient had recovered from his hepatic encephalopathy within a week to 10 days after stopping rhIL-15 with slower improvement of his liver function tests. When the patient was taken off protocol due to disease progression his scans demonstrated multiple hepatic and mesenteric masses in excess of 10 cm in diameter and large volume replacement of his liver with the patient's malignancy. Patient #21's grade 5 SAE was problematic on many levels including the ultimate inability to define the primary pathophysiology that occurred with this event. The patient was assessed as initially having an osmotic diarrhea related to change in her G-tube oral supplementation that was potentiated by the empiric addition of ampicillin/sulbactam (Unasyn) that frequently causes antibiotic associated enteritis. Prior experience in NCI and

extramural rhIL-15 clinical trials had not shown any real evidence of IL-15 related diarrhea or potentiation of diarrhea due to another cause despite NHP toxicology results that demonstrated diarrhea in the macaques. The diffuse patchy ischemia seen in the patient's stomach, small and large bowel at laparotomy infers that rhIL-15 may have potentiated localized inflammation that compromised small vessel blood flow. We believe that both of these patients represent very unique situations that can be avoided by additional changes in eligibility, dose adjustment algorithm and DLT criteria to allow evaluation of rhIL-15 above 2 mcg/kg in a 5-day treatment schedule with sufficient safety margin.

The effort to identify the optimal regimen for any new immunotherapeutic requires clinical evaluation of the various routes of administration and different schedules. Most of the murine preclinical IL-15 studies did not perform comparative examination of different schedules, routes of administration or repeat cycles of treatment that could have helped identify the most promising route and schedules of rhIL-15 to be assessed in the initial clinical trials, and our recent murine experiments were also not comprehensive. While the IVB and CIV toxicology studies were performed sequentially, there are data that allows some comparative deductions. Preclinical data from the initial NHP toxicology experiment evaluating IVB rhIL-15 showed the increase in absolute numbers of lymphocytes was most notable for NK and $CD8^+$ cells predominantly in the stem, central and effector memory subsets. Early data from the current IVB clinical trial has demonstrated less consistent expansion of $CD8^+$ cells. Comparison of the immune activation derived from IVB and CIV dosing in these NHP toxicology experiments showed that CIV produced a significantly greater ALC expansion (2.5-fold vs.10 to 25-fold) after a single cycle of rhIL-15 treatment.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Age ≥ 18 years.

2.1.1.2 Patients must have histologically confirmed (by the NCI Pathology Department) solid tumor malignancy or lymphoma that is metastatic or unresectable and for which standard curative or palliative measures do not exist or are associated with minimal patient survival benefit (as defined by the Lymphoid Malignancies Branch physicians) or if the patient refuses standard of care treatment. Enrollment of patients with tumors that can be safely biopsied is encouraged.

2.1.1.3 Patients must have evaluable or measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan. See Section **6.2** for the evaluation of measurable disease.

2.1.1.4 Patients must have recovered to ≤ grade 1 CTCAE v4 from toxicity of prior chemotherapy or biologic therapy and must not have had prior chemotherapy or biologic therapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C, 8 weeks for UCN-01).

2.1.1.5 Patients on bisphosphonates for any cancer or on hormone therapy for prostate cancer will not need to discontinue this therapy to be eligible. However, patients with prostate cancer will need to have metastatic prostate cancer that has progressed despite hormonal therapy. Castrate testosterone levels occur within hours after castration and within 2 to 3 weeks of a luteinizing hormone-releasing hormone agonist. The current standard is to continue androgen suppression despite progressive disease.

2.1.1.6 DLCO/VA and FEV-1.0 ≥ 60% of predicted on pulmonary function tests.

2.1.1.7 Serum creatinine of ≤ 1.5 X the upper limit of normal.

2.1.1.8 AST and ALT < 2.5 x the upper limit of normal.

2.1.1.9 Absolute neutrophil count ≥ 1,500/mm^3 and platelets ≥ 100,000/mm^3.

2.1.1.10 Karnofsky performance status ≥ 70% or ECOG ≤ 1 (see **APPENDIX A**)

2.1.1.11 Subjects with inactive central nervous system (CNS) metastasis are eligible. Inactive CNS metastasis is defined as: no signs of cerebral edema after successful definitive treatment of brain metastases (surgical resection, whole brain irradiation, stereotactic radiation therapy, or a combination of these) with stable or improved radiographic appearance on MRI scan at least 1 month after completion of treatment.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who have received any systemic corticosteroid therapy within 3 weeks prior to the start of therapy with the exception of physiological replacement doses

of cortisone acetate or equivalent.

2.1.2.2 Patients who have received any cytotoxic therapy, immunotherapy, antitumor vaccines, monoclonal antibodies or major surgery in the 4 weeks prior to the start of the study.

2.1.2.3 Life expectancy of less than 3 months.

2.1.2.4 Patients with more than 30% replacement of hepatic parenchyma by tumor or any history of drug related hepatic encephalopathy.

2.1.2.5 History of complex ventricular or supraventricular arrhythmias.

2.1.2.6 Documented HIV, active bacterial infections, active or chronic hepatitis B, or hepatitis C.

- A positive hepatitis B serology indicative of previous immunization (i.e., HBsAb positive and HBc Ab negative), or a fully resolved acute hepatitis B infection is not an exclusion criterion.
- A positive hepatitis C serology is an exclusion criterion.

2.1.2.7 Concurrent anticancer therapy (including other investigational agents), with the exception of hormone therapy for prostate cancer (see Section **2.1.1.5**).

2.1.2.8 Active CNS metastasis (inactive CNS metastasis is defined in Section **2.1.1.11**).

2.1.2.9 History of severe asthma or presently on chronic inhaled corticosteroid medications (patients with a history of mild asthma controlled with inhaled bronchodilators are eligible).

2.1.2.10 History of autoimmune disease, with the exception of an autoimmune event associated with prior ipilimumab (anti-CTLA-4) therapy that has been completely resolved for more than 4 weeks.

2.1.2.11 Inability or refusal to practice effective contraception during therapy or the presence of pregnancy or active breastfeeding (men and women of childbearing potential must use an effective method of birth control or abstinence during treatment and for 4 months after completion of treatment).

2.1.2.12 Cognitive impairment, history of medical or psychiatric disease, other uncontrolled intercurrent illness, active substance abuse, or social circumstances, which in the view of the Principal Investigator (PI), would preclude safe treatment or the ability to give informed consent.

2.2 SCREENING EVALUATION

Screening evaluation must be performed to determine eligibility within 28 days prior to patient receiving any study intervention, except as indicated below.

2.2.1 Complete medical history and physical examination with vital signs and documentation of all measurable or evaluable abnormalities.

2.2.2 Computerized tomography (CT) scan of the chest, abdomen, and pelvis. (CT and/or MRI films from outside institution performed within 28 days prior to enrollment may be used to determine eligibility. Same films may be used as baseline if done within 28 days prior to starting treatment.)

2.2.3 MRI or contrast-enhanced CT scan of the brain for melanoma subjects or subjects with complaints and/or physical findings suspicious for occult CNS metastasis.

2.2.4 Documentation of Karnofsky Performance Score ≥ 70% or ECOG ≤ 1 (see **APPENDIX A**).

2.2.5 Verification that the NCI Laboratory of Pathology has confirmed the histology prior to patient registration (No Time Frame). Pathological confirmation of diagnosis can be carried out on available slides from either the primary diagnostic biopsy or subsequent biopsies performed either at the Clinical Center or outside institutions. If necessary, the specimen blocks will be obtained for special stains and other evaluation as deemed necessary to confirm the diagnosis of cancer. If the original pathological material or other diagnostic biopsies are not available, or if there is uncertainty concerning the diagnosis, a repeat biopsy may be undertaken to confirm the diagnosis of cancer.

2.2.6 Complete blood count (CBC) with differential and platelet count Acute Care Panel (sodium, potassium, chloride, CO_2, glucose, BUN, creatinine); Hepatic panel (alkaline phosphatase, ALT, AST, total and direct bilirubin), LDH, Mineral panel (serum calcium, phosphate, magnesium and albumin) and ammonia level.

2.2.7 Serum pregnancy test in women of childbearing potential.

2.2.8 Thyroid function tests to include a serum thyroxine (T4) and thyroid-stimulating hormone (TSH).

2.2.9 Anti-nuclear antibodies (ANA), rheumatoid factor (RF) and anti-thyroid antibodies

2.2.10 Urinalysis.

2.2.11 12 lead EKG.

2.2.12 Hepatitis B Surface Antigen, Hepatitis B Surface Antibody, Hepatitis B core Antibody and Hepatitis C: HIV 1/2 serology.

2.2.13 Pulmonary function tests with DLCO.

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office at ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the subject on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4 BASELINE EVALUATION

The following tests and procedures will be done within 28 days of initiating treatment with rhIL-15 (except as marked below). If baseline tests were already performed and meet the required time frames, they will not need to be repeated.

2.4.1 Baseline history with documentation of sites of disease, physical examination, vital signs and performance score.

2.4.2 Serum pregnancy test in women of childbearing potential must be repeated within 3 days prior to starting study drug.

2.4.3 CBC with differential, Acute Care Panel, Hepatic Panel, LDH and Mineral Panel must be repeated if not done within 2 weeks of starting study drug.

2.4.4 Reticulocyte Count

2.4.5 Serum immunoglobulin levels

2.4.6 Troponin T or I, based on availability of test in Department of Lab Medicine (DLM)

2.4.7 HLA typing (A, B, C, DR, DQ)

2.4.8 Serum tumor marker (PSA, CEA, and CA125) assessment if applicable (within 7 days prior to initiating treatment with rhIL-15)

2.4.9 CT scan of the chest, abdomen and pelvis, or MRI if CT scan is not sufficient or contraindicated

2.4.10 Other imaging studies may be done to document disease as appropriate and at the direction of the PI.

2.4.11 Baseline flow cytometry to assess lymphocyte sets and subsets (see Section **5.1.2**):

2.4.11.1 One 10-mL lavender-top tubes to Clinical Center Dept. of Lab. Medicine for Lymphocyte Phenotype IL-15 FACS panel.

2.4.11.2 Two lavender-top tubes to the Clinical Support Laboratory in Frederick for special FACS analysis.

2.4.12 One 4-mL Serum Separator Tube (SST) tube to the Clinical Support Laboratory in Frederick for inflammatory marker and cytokine analysis (see Section **5.1.3**).

2.4.13 Optional Tumor Biopsy (see Section **5.1.5**)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase I open-label, non-randomized, dose-escalation safety trial of CIV rhIL-15 that will enroll 41 to 47 evaluable patients with metastatic unresectable cancers for which curative or palliative measures either do not exist or are not associated with a survival advantage.

Patients will receive CIV rhIL-15 for 10 consecutive days (240 hours) and will be assigned to a dose level sequentially based on their order of entry into the study. The starting dose will be 0.1 mcg/kg/day and in the absence of significant toxicities, dose escalation will proceed to evaluate dose levels of 0.25, 0.5, 1, 2 and 4 mcg/kg/day. Cycles 1 and 2 will be 42 days in length, but all subsequent treatment cycles will be 28 days in length with an additional 7 days on either schedule allowed for reasons other than recovery for treatment-related toxicities. Subjects will have to demonstrate an absence of anti-IL-15 antibodies in their post-treatment (day 12 and week 4) assessments before additional cycles of rhIL-15 can be initiated.

Radiologic reassessments for 10-day treatment cycle patients will be performed during week 4 (days 26 to 35) of Cycles 1 and 2 and at off-treatment and off-study visits.

After treatment of patients in the expansion cohort for the 10-day schedule is completed, new cohorts of 3 to 6 patients each will begin treatment with the 5-day (120 hour) schedule at dose levels of 3, 4 or 5 mcg/kg/day to define the MTD for this shorter CIV rhIL-15 regimen. All treatment cycles for these cohorts will be 21 days in length.

As of April 2016, twenty-four evaluable patients have enrolled on Dose Levels 1-6; with 2 more to be enrolled in Dose Level 6 after Amendment G. When the study is re-opened for enrollment, an additional 17-23 evaluable patients will be enrolled.

A total of 9 patients will potentially be treated at the MTD or maximum administered dose for both schedules to better characterize the clinical activity of CIV rhIL-15 in this patient population.

Radiologic reassessments for both the 10day and 5-day dosing cohorts will be performed after every other cycle (2, 4, 6, 8 etc.) including the subjects that go onto to receive additional cycles of treatment.

Patients receiving the 10-day treatment schedule without evidence of an ongoing response after any 2 consecutive cycles of treatment will discontinue rhIL-15 and be followed until disease progression is documented or they go off study for another reason. (Ongoing response is defined as: > 15% decrease in sum of marker lesions and/or improvement or disappearance of some non-measurable lesions and/or > 10% decrease in tumor markers.) Patients receiving the 5-day treatment schedule will have 4 cycles of treatment to demonstrate responsiveness to rhIL-15, so that patients are given an equal number of rhIL-15 doses to confirm the treatment has sufficient activity to justify more prolonged treatment.

Patients who demonstrate a complete response (CR) to treatment will receive 2 additional cycles after the cycle where the CR is first documented.

Patients whose best response after 2 consecutive cycles of treatment is stable disease and those who stop treatment due to toxicity will be followed with routine exams and restaging x-rays (every 60 days x 3 and then quarterly) unless progressive disease is documented or they begin another treatment for their cancer.

3.2 DOSE-LIMITING TOXICITY

There are some well-described, clinically inconsequential side effects associated with immunotherapy that are addressed differently than with other types of cancer therapies and affect the consideration of DLTs. Patients receiving rh cytokines often have an initial decrease in their total white blood cell (WBC) counts, significant lymphopenia and occasionally some degree of neutropenia early in the course of their treatment (66, 67). Unlike cytopenias noted in patients receiving cytotoxic chemotherapies, these drops are usually transient and presumed to be related to increased margination (adherence to blood vessel endothelium) or alterations in trafficking that reduces the number of circulating cells rather than the destruction of the mature effector and bone marrow precursor cells (68). The impediment to normal trafficking (chemotaxis) of various classes of leucocytes best described for patients treated with HD IL-2 marginally increases the risk for infections, especially central catheter–associated bacteremia; but the rate of infections in cytokine-treated patients is substantially less than leucopenic and neutropenic chemotherapy-treated patients (69).

As such, rh cytokine treatment resulting in grade 3 or 4 cytopenias, especially lymphopenia, in the absence of signs of infection is usually not interrupted. Less commonly, patients receiving

activated T-cells and cytokine therapy will have a significant leukocytosis (67). Even though these cells are activated and generally large lymphocytes, toxicities seen in leukemic patients with elevated WBC counts are not encountered and therapy is not interrupted based purely on an arbitrary WBC number. In the completed IVB trial and in this protocol, we have observed most commonly during the first 7 days of treatment transient increases in liver function tests (alkaline phosphatase, alanine aminotransferase [ALT] and aspartate aminotransferase [AST] which usually begin to improve while the subject continues treatment and have not been associated with any clinical signs of hepatotoxicity. During the IVB trial, treatment was discontinued for grade 3 elevations (> 5 times ULN) of AST and/ALT in 2 subjects treated at the 1 mcg/kg dose level who otherwise were tolerating their treatment very well. Some subjects previously treated in this protocol have had less significant elevations that have also resolved without incident, DLTs or clinical sequelae. HDIL-2 subjects commonly have abnormalities of their LFTs in many occasions much more abnormal than we have observed in the rhIL-15 subjects and continue their treatment (59, 60). Similar transient increases which resolve spontaneously most often with continued rhIL-15 or in all cases after treatment is stopped have also not been associated clinical hepatotoxicity with the one exception discussed in Section **1.2.1.6**. We believe that the clinical experience with rhIL-15 in all three trials with the additional limitation on degree of hepatic tumor replacement allows continuation of the DLT exception with regards to ALT, AST, alkaline phosphatase, total and direct bilirubin.

3.2.1 DLT will be defined as follows:
Grade 2 diarrhea lasting more than 24 hours or any grade 3 or 4 toxicity, if deemed possibly, probably or definitely related to the study drug by the PI during the first cycle of treatment, **with the following exceptions:**

Hematologic exceptions:

- Grade 3 or 4 lymphopenia
  - rhIL-15 will be continued in the event of asymptomatic grade 3 or 4 lymphopenia, unless there are clinical signs of significant infection (persistent fevers, labile blood pressure, localized complaints or findings on physical exam, hypoxia or organ dysfunction).
- Grade 3 granulocytopenia
  - rhIL-15 will be continued in the event of grade 3 granulocytopenia unless there are clinical signs indicating a significant infection, as listed above.
- Grade 3 leukocytosis (WBC > 100, 000/mm^3) in the absence of signs of leukostasis or other toxicities possibly related to the expansion of activated cells.

Non-hematologic exceptions:

- Transient (< 24 hours) grade 3 hypoalbuminemia, hypokalemia, hypomagnesemia, hyponatremia or hypophosphatemia which responds to medical intervention. rhIL-15 will be continued while the metabolic abnormalities are corrected by intravenous and oral supplementation.
- Non-sustained (< 7 days) grade 3 liver function test (ALT, AST, alkaline phosphatase, total or direct bilirubin) abnormalities in the absence of clinical signs of hepatic dysfunction (lethargy, confusion, anorexia, pruritus, tremor). rhIL-15 will be continued as long as these abnormalities do not persist more than 7 days or the patient does not exhibit findings consistent with hepatic toxicity.

3.3 DOSE ESCALATION

Dose escalation will proceed in cohorts of 3 to 6 patients. Patients will not begin treatment at the next higher dose level until all patients treated at the previous dose level have reached day 21 of the protocol, recovered from any clinical or laboratory toxicities and would be able to initiate another cycle of treatment unless restaging has demonstrated progression of their disease). The MTD is the dose level at which no more than 1 of up to 6 patients experience DLT, and the dose below that at which at least 2 (of ≤ 6) patients have DLT as a result of the drug. If a patient did not experience DLT and did not finish 1 cycle of treatment (42 days), he or she will not be evaluable for determination of the MTD and will be replaced in the dose level.

Dose escalation will follow the rules outlined in the Table below.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter up to 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to 3 additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to 3 additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

The MTD has already been defined for patients treated with the 10-day regimen. For patients treated with the 5-day regimen dose escalation will continue through the 5 mcg/kg/day dose level in the absence of ≥ 2 DLTs for the patients treated for any of the dose levels being evaluated for the 5-day regimen. If an MTD is not identified up to 9 patients may be treated at the maximum administered dose (MAD) of 5 mcg/kg/day to better evaluate the therapeutic potential of rhIL-15 given by this schedule.

3.4 DRUG ADMINISTRATION

For all dose levels, the dose of rhIL-15 will be diluted in the appropriate volume of 0.1% human serum albumin (HSA) in 5% dextrose in water, USP (D5W) to reach a final rhIL-15 concentration of 1 mcg/mL. The rhIL-15 treatment will be administered to the patient by continuous 24-hour intravenous infusion (CIV) using portable ambulatory pump on the inpatient unit for either a total of 240 or 120 hours depending on the treatment cohort. Treatment with rhIL-15 will begin within 4 hours of preparation of the infusion bag and each infusion bag must be completed within 24 hours from the time drug administration begins. If the infusion bag is not empty at the 24-hour time point, the remaining solution will be discarded and the next infusion bag will be started. **The 240- or 120-hour infusion** time frame **will not be extended.**

The rhIL-15 infusion solution is stable at a concentration of 1 mcg/mL with 0.1% HSA for 4 hours at controlled room temperature (15°C–30°C) prior to initiation of the 24-hour infusion or 24 hours at 2-8º C prior to initiation of the 24-hour infusion. This stability information was previously documented by the Biopharmaceutical Development Program (BDP) of Leidos Biomedical Research, Inc.-Frederick, the drug manufacturer.

The rhIL-15 will be infused through a PICC or another appropriate CVAD.

Samples for PK assessments must **not** be drawn from the PICC or CVAD through which the rhIL-15 is infused. If feasible, venous access will be placed at 2 locations **NOT in the same extremity** during cycle 1. This will prevent contamination of the blood draws with residual rhIL-15 present in the catheter or double lumen catheters with distal openings in close proximity.

In Cycle 2 and beyond, the IL-15 infusion may be put on hold for 5-15 mins to collect routine (non-PK) daily labs.

3.5 DOSE MODIFICATIONS

Since this is a phase I study intending to define the MTD, there will be no dose modifications or interruption of treatment during the first cycle of treatment for the 5-day dosing cohorts. **The rhIL-15 infusion maybe interrupted for up to 24 consecutive hours for reasons other than DLTs without bearing on the patient's continued treatment during subsequent treatment cycles for patients treated with either schedule. Interruptions of the infusion will not be made up by extending the 240- or 120-hour infusion and there will be no change in the date and time the 10- or 5-day infusion is terminated unless the treatment is stopped early for safety reasons.** Patients with evidence of clinical benefit at restaging may have interruptions or early termination of their CIV rhIL-15 during later retreatment cycles for non-DLT events to improve tolerability of the treatment without forfeiting the possibility of subsequent treatment cycles. Stoppage of less than 15 minutes occurring during the daily change of the rhIL-15 infusion device will not be considered an interruption.

3.6 MONITORING DURING THERAPY

The following tests and procedures will be performed during all treatment cycles as indicated in Section **3.8**, Study Calendars.

3.6.1 Acute Care, Hepatic and Mineral Panels, ammonia level and LDH

3.6.2 CBC with differential

3.6.3 Troponin T or I, based on availability of test in DLM

3.6.4 ANA, RF, anti-thyroid antibody and thyroid function tests: TSH and T4

3.6.5 EKG

3.6.6 Percutaneously Inserted Central Catheter (PICC) or another appropriate central vascular access device (CVAD) will be inserted from Day -7 to Day 1 of each treatment cycle for infusion of rhIL-15. An additional PICC, CVAD or a peripheral venous catheter may be inserted and used for drawing multiple blood samples as needed. Samples for PK assessments must **not** be drawn from the CVAD through which the rhIL-15 is infused.

3.6.7 Radiographic Evaluation and Laboratory Assessments for Clinical Responses

3.6.8 Other Studies

Patients may have additional laboratory or radiological studies performed as clinically indicated, in particular if there were a suspicion of disease progression that would warrant an urgent therapeutic intervention.

3.6.9 Vital signs

3.6.10 Fluid intake and urine output (I/O)

3.6.11 Weight

3.6.12 Interval history and physical examination

3.7 MONITORING DURING FOLLOW-UP PERIOD (AFTER COMPLETION OF TREATMENT)

The following tests and procedures will be performed during the follow-up portion of the study, as indicated in Section **3.8,** Study Calendars.

3.7.1 Vital signs

3.7.2 Weight

3.7.3 History and physical examination, including documentation of performance status

3.7.4 ANA, RF, anti-thyroid antibody and thyroid function tests: TSH and T4

3.7.5 Acute Care, Hepatic and Mineral Panels and LDH

3.7.6 CBC with differential

3.7.7 Radiographic evaluation and laboratory assessments for clinical responses

3.8 STUDY CALENDARS

3.8.1 10 Day Schedule

Procedure	Pre-treatment		Day on Treatment (For All Cycles[1])											During-treatment			Follow-Up Period[3]	End of Study[4]
	Screening	Baseline[16]	1	2	3	4	5	6	7	8	9	10	11	12[2]	21-27	28-34		
CIV rhIL-15 treatment[5]			x	x	x	x	x	x	x	x	x	x	x					
Pathology confirmed	x																	
Performance status	x	x	x							x						x	x	x
History & physical exam[6]	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x
Vital Signs[7] and Weight	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x	x
Acute Care, Hepatic and Mineral Panels and LDH	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x
Ammonia level	x		x											x				
I/Os			x	x	x	x	x	x	x	x	x	x	x	x				
CBC w/differential,	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Reticulocyte Count		x																
Hepatitis and HIV serology	x																	
Thyroid function panel	x																x	
Troponin T		x	x					x						x		x		
Urinalysis	x																	
EKG	x		x									x				x		
Pulmonary function tests	x																	
HLA (A, B, C, DR, DQ) typing		x																
Tumor imaging and serum tumor markers[8]	x	x														x[8]	x	
Serum βHCG[9]	x		x															
ANA, RF and anti-thyroid antibodies	x																x	
Pharmacokinetics (PK)[10]			x	x	x				x	x	x	x	x	x				
Serum immunoglobulin levels		x																
Flow cytometry (Fleisher Lab)[11]		x	x							x				x	x	x		
Flow cytometry (Roederer Lab)[12]		x		x						x				x	x	x		
Cytokine and MesoScale inflammatory cytokines[13]			x	x						x				x				
Anti-IL-15 antibodies[14]			x											x		x	x	
Optional Tumor Biopsy [15]		x									x							

1. Cycles 1 and 2 for the 10-day dosing schedule will be 42 days in length, but subsequent cycles will be 28 days in length with all cycles allowing an additional 7 days allowed for reasons other than recovery for treatment-related toxicities.
2. Day 12 tests and procedures only occur on Cycle 1.
3. Patients whose best response after 2 consecutive cycles of treatment is stable disease will be followed with routine exams and restaging scans: Every 60 days x 3; then quarterly for up to 2 years after starting treatment unless progressive disease is documented, or they begin another treatment for their cancer.
4. Denotes testing to be performed at an end of study visit (just prior to the patient being taken off study), if feasible.
5. Because the treatment is CIV for 240 hours, the day 10 treatment concludes on day 11.
6. Interval history during inpatient treatment period.
7. Vital signs, including heart rate, blood pressure, respiration, temperature and arterial oxygen saturation (SO2) by transcutaneous pulse-oximeter.
    a. Every 4 hours during treatment days 1 through 11 (SO2 every 8 hours)
    b. Every 8 hours on other inpatient days
    c. Once at outpatient clinic follow-up visits on day 28.
    d. Off treatment visit
    e. End of study visit.
8. Repeat radiologic studies to assess response week 4 of cycles 1 and 2. Subjects that go onto to receive additional cycles of treatment will have restaging after every other cycles (4, 6, 8 etc.) and may be restaged earlier if the subject has complaints or findings that indicate progressive disease. Subjects with evidence of a partial or complete response (RECIST criteria) may undergo a confirmatory scan at least 4 weeks after the scan which first met the criteria for a clinical response. Patients with tumors known to have tumor markers (PSA, CEA, and CA125) will have the serum level of these markers assessed at the time of their radiographic restaging.
9. Pretreatment on Day 1 or within 3 days of starting treatment in women of child bearing potential.
10. PKs are collected on Cycle 1 only. Samples are collected in 4 mL SST tubes at the time points listed in Section **5.1.1**. These samples will be held at room temperature until picked up by the Figg laboratory for processing (pager 102-11964 for pickup). See Section **5.1** for additional details.
11. One 10 mL lavender-top tube will be drawn for Lymphocyte Phenotype IL-15 FACS analysis. If the Immunology Section Laboratory is unable to perform this analysis on the specified days, this assessment maybe omitted or replaced with standard TBNK panel. See Section **5.1.2** for additional details:
    a. At baseline (need to perform twice before initiating study drug if at all possible.)
    b. Day 1: prior to the start of the CIV rhIL-15 infusion
    c. Days 8 and 12 drawn with the routine AM laboratories
    d. Days 21and 28: at the same time as the outpatient blood draws.
12. Two 10 mL lavender-top tubes will be drawn for the Roederer Lab (NIAID) to perform special FACS analysis. See Section **5.1.2** for additional details:
    a. At baseline (OK to draw on Day 1 or up to 2 weeks prior to initiating study drug.)
    b. Day 2: 24 hours after starting the CIV rhIL-15 infusion
    c. Days 8 and 12: Samples may be drawn with the routine AM laboratories
    d. Days 21and 28: Samples may be drawn at the same time as the outpatient blood draws.
13. Cytokine and MesoScale inflammatory cytokines are collected on cycle 1 only: Samples to assess for IL-2Rα IL-18, MIP2, MIP1α, TNFα and MesoScale Discovery Multiplex Pro-Inflammatory Cytokines (IL-1β, IL-12p70, Interferon γ, IL-6, IL-8 and IL-10) will be obtained from the same SST tubes drawn for PK time points drawn at the time listed above in # 10. See Section **5.1.3** for additional details.
14. For all samples: Assessments that fall on a Friday, Saturday or Sunday due to an individual patient's treatment schedule will be shifted forward or backward to the nearest day that these laboratories are able to process the time sample.
15. Optional biopsies will be performed (if patient agrees) pre-treatment and one subsequent time between Day 8 and Day 21 as determined by clinical response and/or changes in lymphocyte subsets.
16. If baseline tests were already performed and meet the required time frames, they will not need to be repeated.

3.8.2 5 Day Schedule

Procedure	**Pre-treatment**		**Days During-treatment[1]**								**After treatment**		
	Screening	Baseline[15]	1	2	3	4	5	6	8	21±4[1]	End of Treatment[16]	Follow-Up Period[2]	End of Study[3]
CIV rhIL-15 treatment[4]			x	x	x	x	x	x[4]					
Pathology confirmed	x												
Performance status	x	x	x						x		x	x	x
History & physical exam[5]	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital Signs[6] and Weight	x	x	x	x	x	x	x	x	x	x	x	x	x
NIH Advance Directives Form[17]													
Acute Care, Hepatic and Mineral Panels and LDH	x	x	x	x	x	x	x	x	x	x	x	x	x
Ammonia level	x		x						x				
I/Os			x	x	x	x	x	x					
CBC w/differential	x	x	x	x	x	x	x	x	x	x	x	x	x
Reticulocyte Count		x											
Hepatitis and HIV serology	x												
Thyroid function tests: TSH and T4	x										x	x	
Troponin T or I, based on availability of test in DLM		x	x										
Urinalysis	x												
EKG	x		x										
Pulmonary function tests	x												
HLA (A, B, C, DR, DQ) typing		x											
Tumor imaging and serum tumor markers[7]	x	x										x	
Serum βHCG[8]	x		x										
ANA, RF and anti-thyroid antibodies	x											x	
Pharmacokinetics (PK)[9]			x	x	x	x	x	x					
Serum immunoglobulin levels		x											
Flow cytometry (Fleisher Lab)[10]		x	x						x	x			
Flow cytometry (Roederer Lab)[11]		x	x						x	x			
Cytokine and MesoScale inflammatory cytokines[12]			x	x	x	x	x	x	x	x			
Anti-IL-15 antibodies[13]			x							x		x	
Optional Tumor Biopsies[14]		x							x				

1. All cycles are 21 days in length, but the end of cycle evaluations may be performed ± 4 days from this time point and an additional 7 days allowed for reasons other than recovery for treatment-related toxicities. Cycle 1 day 21 and cycle 2 day 1 maybe the same calendar day for certain patients. This also applies to later cycles where day 21 of a previous cycle falls on the same calendar day as day 1 of the subsequent cycle.
2. Patients whose best response after 4 consecutive cycles of treatment is stable disease will be followed with routine exams and restaging scans: Every 60 days x 3; then quarterly for up to 2 years after starting treatment unless progressive disease is documented, or they begin another treatment for their cancer.
3. Denotes testing to be performed at an end of study visit (just prior to the patient being taken off study), if feasible.
4. Because the treatment is CIV for 120 hours, the day 5 treatment concludes on day 6.
5. Interval history during inpatient treatment period.
6. Vital signs, including heart rate, blood pressure, respiration, temperature and arterial oxygen saturation (SO2) by transcutaneous pulse oximeter.
    a. Every 4 hours ± 1 hour during treatment days 1 through 6 (SO2 every 8 hours ± 1 hour)
    b. Every 8 hours ± 1 hour on other inpatient days
    c. Once at outpatient clinic follow-up visits on days 8 and 21.
    d. Off treatment visit
    e. End of study visit.
7. Repeat radiologic studies to assess response the last week (day 14 through 21) of even numbered cycles (2, 4, 6 and so on) as long as patients meet criteria to continue treatment. Patients with evidence of a partial or complete response (RECIST criteria) may undergo a confirmatory scan at least 4 weeks after the scan which first met the criteria for a clinical response. Patients with tumors known to have tumor markers (PSA, CEA, and CA125) will have the serum level of these markers assessed at the time of their radiographic restaging.
8. Pretreatment on Day 1 or within 3 days of starting treatment in women of child bearing potential.
9. PKs are collected on Cycle 1 only. Samples are collected in 4 mL SST tubes at the time points. These samples will be held at room temperature until picked up by the Figg laboratory (Blood Processing Core) for processing (pager 102-11964 for pickup). See Section **5.1** for additional details.
10. One 10 mL lavender-top tube will be drawn for Lymphocyte Phenotype IL-15 FACS analysis. If the Immunology Section Laboratory is unable to perform this analysis on the specified days, this assessment maybe omitted or replaced with standard TBNK panel. See Section **5.1.2** for additional details:
    a. At baseline (need to perform twice before initiating study drug if at all possible.)
    b. Day 1: prior to the start of the CIV rhIL-15 infusion
    c. Days 8 and 21 drawn with the routine laboratories
11. Two 10 mL lavender-top tubes will be drawn for the Roederer Lab (NIAID) to perform special FACS analysis. See Section **5.1.2** for additional details:
    a. At baseline (need to perform twice before initiating study drug if at all possible.)
    b. Day 1: prior to the start of the CIV rhIL-15 infusion
    c. Days 8 and 21 drawn with the routine laboratories.
12. **Cytokine and MesoScale inflammatory cytokines are collected on cycle 1 only**: Samples to assess for IL-2Rα IL-18, MIP2, MIP1α, TNFα and MesoScale Discovery Multiplex Pro-inflammatory Cytokines (IL-1β, IL-12p70, Interferon γ, IL-6, IL-8 and IL-10) will be obtained from the same SST tubes drawn for PK time points drawn at the time listed in Section **5.1.3.** Refer to this Section for additional details.
13. For all samples: Assessments that fall on a Friday, Saturday or Sunday due to an individual patient's treatment schedule will be shifted forward or backward to the nearest day that these laboratories are able to process the time sample.
14. Optional biopsies will be performed (if patient agrees) pre-treatment and one subsequent time between Day 8 and Day 21 as determined by clinical response and/or changes in lymphocyte subsets.
15. If baseline tests were already performed and meet the required time frames, they will not need to be repeated.
16. End of treatment visit will occur approximately 30 days after the last dose of study drug. If the patient cannot return to the Clinical Center, a request will be made to collect clinical labs from a local physician or laboratory. If this is not possible, patients may be assessed by telephone for symptoms.
17. All subjects will be offered the opportunity to complete an NIH advance directives form (Section **10.3**); preferably done at baseline, but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

3.9 CONCURRENT THERAPY

Concurrent anti-cancer therapy to include immunotherapy, immunosuppressive therapy, radiation therapy or chemotherapy, and administration of systemic corticosteroids except for physiologic replacement doses of cortisone acetate is not permitted while on the study. Initiation of medically indicated, non-oncologic medications such as anti-hypertensive agents or antibiotics for urinary tract infections that do not have significant potential for interaction with the rhIL-15 treatment are allowable with prior approval of the PI.

3.10 CRITERIA FOR REMOVAL FROM THE PROTOCOL THERAPY AND OFF-STUDY CRITERIA

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.10.1 Off-Treatment Criteria

- DLT as defined in Section **3.2**.
- Persistent (> 14 days) treatment-related grade 2 (non-DLT) adverse events.
- Completion of protocol therapy
- Patient requests to be withdrawn from active therapy.
- Disease progression.
- No evidence of clinical response (i.e., patient has stable disease) on radiographic restaging after 2 consecutive cycles of treatment.
- The detection of anti-IL-15 neutralizing antibody serum titer ≥ 500 ng/mL as described in **APPENDIX C** prior to subsequent treatment cycles.
- Evidence the patient has developed auto-immunity that involves a vital organ (heart, kidney, brain, eye, thyroid or adrenal gland, colon, lung). Patients who develop new auto-antibodies without clinical evidence of end organ dysfunction will be seen by the appropriate Internal Medicine subspecialists to assess their risk for developing an autoimmune syndrome.
- Interruption of CIV rhIL-15 treatment for any reason for more than 24 consecutive hours during cycle 1. Stoppage of less than 15 minutes occurring during the daily change of the rhIL-15 infusion device will not be considered an interruption.
- Positive pregnancy test

3.10.2 Off-Study Criteria

- Voluntary withdrawal of consent by the patient to participate in the study
- Patient non-compliance with treatment and research plans
- Initiation of another anticancer treatment
- Any new medical or psychiatric condition that, in the opinion of the PI, precludes the safe continuation of the study
- Death

3.10.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office at

ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

Patients will be followed and managed as clinically appropriate with IV fluids, anti-emetics, anti-pyretics or other standard interventions based on the experience gathered from the ongoing rhIL-15 IVB trial and other biological response modifier therapies.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH, PHARMACOKINETIC AND SPECIAL STUDIES

Please e-mail Julie Barnes at Julie.barnes@nih.gov and Paula Carter (pcartera@mail.nih.gov) at least 24 hours before transporting samples to the (Figg Lab) Blood Processing Core (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.

5.1.1 Pharmacokinetic (PK) Studies

All PK samples will be drawn in 4 mL SST tubes and PK testing will be performed **only during the first treatment cycle**. Labels listing the patient's name, date of birth, date and time of the blood draw will be affixed to all the tubes by the staff person who obtained the samples. Please page 102-11964 (Figg lab) for pick-up. Because each treatment day is a 24 hour infusion, the "day 10 treatment" ends on day 11. Samples will be held at room temperature until picked up by the Figg lab (Blood Processing Core) for processing and shipping of the samples to a Contractor for performance of the assays. At time points where PK and inflammatory marker cytokine or anti-IL-15 antibody analyses coincide (day 1 pretreatment, hours 8, 4, 24 post initiation of infusion, day 8 and 12) one or two 4 mL SST tube will be drawn to decrease total amount of research blood drawn and the serum aliquoted into vials prior to shipment to the respective reference labs. Patient identifiers will be removed prior to shipment per Section **5.2.1**.

Sampling times for the 10-day infusion schedule are as follows (cycle 1 only):

- **Day 1:** Timed samples will be drawn just before beginning treatment (baseline), and at 10 minutes, 1, 2, 4 8 and 12 hours (± 30 minutes) after starting the CIV rhIL-15 infusion.
- **Day 2:** A single timed sample will be drawn 24 hours (±1 hour) after starting the CIV rhIL-15 infusion.
- **Day 3:** A single timed sample will be drawn 48 hours (±2 hours) after starting the CIV rhIL-15 infusion
- **Days 7, through 10:** Timed samples will be drawn each morning with routine AM laboratory samples. The time the sample was obtained must be recorded.
- **Post-treatment day 11 at completion of infusion and day 12:** Timed samples will be drawn at the end of the 240-hour infusion, 10 and 30 minutes, 1, 2, 4 (± 30 minutes) and 24 (± 2 hours) hours (day 12) after termination of the CIV rhIL-15 infusion.

Sampling times for the 5-day infusion schedule are as follows (cycle 1 only):

- **Day 1:** Timed samples will be drawn just before beginning treatment (baseline), and at

10 minutes, 1, 2, 4 8 and 12 hours (± 30 minutes) after starting the CIV rhIL-15 infusion.

- **Day 2:** A single timed sample will be drawn 24 hours (±1 hour) after starting the CIV rhIL-15 infusion.
- **Day 3:** A single timed sample will be drawn 48 hours (±2 hours) after starting the CIV rhIL-15 infusion
- **Days 4 and 5:** Timed samples will be drawn each morning with routine AM laboratory samples. The time the sample was obtained must be recorded.
- **Post-treatment day 6 at completion of infusion:** Timed samples will be drawn at the end of the 120 hour infusion at 10 and 30 minutes, 1, 2, 4 hours (± 30 minutes).

5.1.2 Flow Cytometry Studies for Absolute Numbers and Percentages of Lymphocyte Sets and T-cell Subsets

All FACS samples will be drawn in 10-mL lavender-top tubes and will be performed **during all treatment cycles** as defined in Section **3.8**. Labels listing the patient's name, date of birth, date and time of the blood draw will be affixed to all the tubes by the staff person who obtained the samples. The same FACS panels will be performed by the Fleisher and Roederer laboratories for all time points analyzed.

- **Pre-treatment:**
  - Two lavender-top tubes drawn for special FACS analysis in the Roederer Laboratory (NIAID). Lymphoid Malignancies Branch Clinical Research personnel will arrange for these samples to be sent via courier for cryopreservation to Clinical Support Laboratory 560/11-27, Building 1050, Boyles Street, Frederick, Maryland 21702, telephone number: 301-846-1707 or 301-846-1917).
  - One 10 mL lavender top tube Lymphocyte Phenotype IL-15 FACS panel will be drawn and sent via routine collection routing to the Immunology Laboratory of the NIH Clinical Center 10/2C410 (to the Attn. of Dr. Thomas Fleisher M.D., phone number: 301-496-4879) for CD2, CD3, CD4, CD8, CD8 beta, CD11c, CD14, CD16, CD20, CD25, CD27, CD28, CD38, CD45RA, CD45RO, CD56, CD57, CD80, CD86, CD95, IL-2R/IL-15R beta (CD122), CD127 (IL-7R alpha), CD123, CD132, CD62L, CCR5, CCR7, Ki67, IL-15R alpha, and Foxp3.

5.1.3 Inflammatory Markers and Cytokine Analysis

All samples will be drawn in 4 mL SST tubes. Serum samples for these analyses **only during the first treatment cycle** will be obtained from the PK samples (see Section **5.1.1**). Labels listing the date and time of the blood draw as well as the patient's name will be affixed to all the tubes by the staff person who obtained the samples. Lymphoid Malignancies Branch Clinical Research personnel will arrange for these samples to be sent via courier to (specified above) the Clinical Support Laboratory, for analysis of IL-2Rα, IL-18, MIP2, MIP1α and TNFα and to storage for the MesoScale Discovery pro-inflammatory cytokines assay (IL-1β, IL-12p70, interferon γ, IL-6, IL-8 and IL-10).

5.1.4 Anti-IL-15 Antibodies

All samples will be drawn in 4 mL SST tubes; sampling will be performed for the days listed below **during all treatment cycles**. Labels listing the patient's name, date of birth, date and time of the blood draw will be affixed to all the tubes by the staff person who obtained the samples. For questions, please contact Dr. Figg's Clinical Pharmacology Program (CPP) at 240-760-6180; additionally, for pre-notification of planned samples (at least 24 hours in advance, the Friday

before is preferred) email Julie Barnes (julie.barnes@nih.gov) and Paula Carter (pcartera@mail.nih.gov). After sample collection, please page 102-11964 for immediate pick-up. For any questions regarding sample processing, you may also contact Julie Barnes by e-mail or at 240-760-6044.

Samples will be held at room temperature until picked up by the Figg lab (Blood Processing Core) for processing and shipping of the samples to a Contractor for performance of the assays. Lymphoid Malignancies Branch Clinical Research personnel will arrange for these samples to be sent via courier to Clinical Support Laboratory in Frederick (specified above) for assay of the anti-IL-15 antibodies.

Sampling times for the 10-day infusion schedule are as follows:

- **Day 1 pre-treatment, days 12, 28 and off-treatment visit:** One SST tube will be drawn to assess for the formation of anti-IL-15 antibodies.
- Patients will have to demonstrate an absence of anti-IL-15 antibodies in their post-treatment (days 12 and 28) assessments before additional cycles of rhIL-15 can be initiated.

Sampling times for the 5-day infusion schedule are as follows:

- **Day 1 pre-treatment, days 6, 21, day 1 of subsequent cycles and off-treatment visit:** One SST tube will be drawn to assess for the formation of anti-IL-15 antibodies.
- Patients will have to demonstrate an absence of anti-IL-15 antibodies in their post-treatment assessments before additional cycles of rhIL-15 can be initiated.

5.1.5 Optional Tumor Biopsies

Subjects with easily accessible superficial tumor deposits will be asked to sign a separate consent for core biopsies that would be performed by the Department of Radiology and Imaging Sciences' Interventional Radiologists (IR) and evaluated by the NCI Pathology Department at two separate time points [pre-treatment and after treatment has started (anytime from Day 8 to Day 21)] to assess the dynamics of T-cell infiltration and changes in gene expression associated with rhIL-15 treatment. The biopsies should be processed as follows:

- LYMB laboratory staff will provide sample tubes with an adequate amount of RNAlater® for 1 or 2 cores or an excisional biopsy. After the biopsy material is placed in RNAlater®, the specimen will be refrigerated for 12 to 72 hours in room 4B40 prior to being sent via courier to the Clinical Support Laboratory in Frederick for cataloging and long-term storage at -80°C.
- A separate core biopsy (18g) will also be obtained. This sample will be transported to the NCI Pathology Department for confirmation that the specimen contains tumor and for performing routine histologic and immunohistochemical analyses. Alternatively, if fine needle biopsy is performed, the sample will be transported to Cytology.
- If excisional biopsy is performed, Dr. Conlon will view the size of the specimen to make sure an adequate volume of RNAlater® is used to breakdown tissue/denature proteins and decide if there is an adequate amount of tissue to perform immunohistochemistry.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.2.1 Procedures for storage and disposition of serum specimens at the Clinical Support Laboratory, Leidos Biomedical Research, Inc.in Frederick, MD:

The Clinical Support Laboratory, Leidos Biomedical Research, Inc. processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. All laboratory personnel with access to patient information annually complete the NIH online course in Protection of Human Subjects. The laboratory is CLIA certified for CD4 immunophenotyping and all laboratory areas operate under a Quality Assurance Plan with documented Standard Operating Procedures that are reviewed annually. Laboratory personnel are assessed for competency prior to being permitted to work with patient samples. Efforts to ensure protection of patient information include:

- The laboratory is located in a controlled-access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to patient samples processed by the laboratory.
- The database resides on a dedicated program server that is kept in a central, locked computer facility.
- The facility is supported by two IT specialists who maintain up to date security features including virus and firewall protection.
- Program access is limited to specified computers as designated by the laboratory director. Each of these computers has a password restricted login screen.
- The database sample entry program itself is accessed through a password protected entry screen.
- The database program has different levels of access approval to limit unauthorized changes to specimen records and the program maintains a sample history.
- Upon specimen receipt, each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID.
- Inventory information will be stored at the vial level and each vial will be labeled with both a sample ID and a vial sequence number.
- Vial labels do not contain any personal identifier information.
- Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long-term storage.
- Access to stored clinical samples is restricted. Investigators establish sample collections under “Source Codes” and the investigator responsible for the collections, typically the protocol Principal Investigator, specifies who has access to the collection.
- Specific permissions will be required to view, input or withdraw samples from a collection. Sample withdrawal requests submitted to approved laboratory staff by anyone other than the repository source code owner are submitted to the source code owner for approval. The repository facility will also notify the Source Code holder of any submitted requests for sample withdrawal.
- It is the responsibility of the Source Code holder (generally the NCI Principal Investigator) to ensure that samples requested and approved for withdrawal are being used in a manner consistent with IRB approval.

- The Clinical Support Laboratory in Frederick does perform testing services that may be requested by clinical investigators including, but not limited to, immunophenotyping by flow cytometry and cytokine testing using ELISA or multiplex platforms.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process.
- The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

5.2.2 Sample Handling, Data Collection and Sample Disposition for the Blood Processing Core (Figg Lab)

- The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.
- All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (a.k.a. LabSamples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined BPC personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All BPC personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.
- LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).
- Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.
- Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.2.3 Disposition of samples

- All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples

to the NCI IRB as soon as he is made aware of such loss

- Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document and the proposed research has undergone prospective IRB review and approval. If new risks are associated with the research (e.g., analysis of germline genetic mutations), the Principal Investigator must amend the protocol and obtain informed consent from all research subjects.
- Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples provided they have an IRB-approved protocol and subject consent. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.
- Samples will be stored for future use following completion of the study in only those subjects who have consented to do so in the Optional Studies section of the Consent Document.
- If, at any time, a subject withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the subject, if so requested), and reported as such to the IRB.
- The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for at least 30 days after removal from study treatment or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention

- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.2 RESPONSE CRITERIA

6.2.1 Radiographic Evaluation and Laboratory Assessments for Clinical Responses

- All patients will have repeat baseline radiologic studies to assess marker lesions (measurable) and evaluable lesions (non-measurable) performed on day 28 ± 3 days of each treatment cycle for comparison to their pre-treatment studies.
- Patients with evidence of a partial or complete response (RECIST criteria) may undergo a confirmatory scan that will be performed at least 4 weeks after the scan which first met the criteria for a clinical response to rhIL-15 treatment.
- Appropriate patients with tumors known to have tumor markers (PSA, CEA, and CA125) will have the serum level of these markers assessed at the time of their radiographic restaging to gain further insight into the potential efficacy of rhIL-15 treatment.

6.2.2 Solid Tumors

Response and progression for all solid tumors with the exception of non-Hodgkin's lymphomas will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) (70). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.2.2.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with rhIL-15.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.2.2.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately

measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.2.2.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in

complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published (71-73). In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer (74).

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.2.2.4 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more

new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

6.2.2.5 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.2.2.6 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	
* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.				

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
**	Only for non-randomized trials with response as primary endpoint.			
***	In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.			
Note:	Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.			

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.2.2.7 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.2.3 Non-Hodgkin's Lymphoma

Response and progression for non-Hodgkin's lymphoma will be evaluated in this study using the International Working Group Recommendations (75).

Complete Response (CR) requires the following:

- Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.
- Typically FDG-avid lymphoma: in patients with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.

- Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed on CT to normal size (≤1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to ≤1.0 cm in their short axis after treatment.
- The spleen and/or liver, if considered enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or causes other than lymphoma.
- If the bone marrow was involved by lymphoma before treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of > 20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but that demonstrates a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.

Partial Response (PR) requires:

- At least a 50% decrease in sum of the product of the diameters (SPD) of up to six of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following: they should be clearly measurable in at least 2 perpendicular dimensions; if possible they should be from disparate regions of the body; and they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase should be observed in the size of other nodes, liver, or spleen.
- Splenic and hepatic nodules must regress by ≥50% in their SPD or, for single nodules, in the greatest transverse diameter.
- With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and no measurable disease should be present.
- Bone marrow assessment is irrelevant for determination of a PR if the sample was positive before treatment. However, if positive, the cell type should be specified (e.g., large-cell lymphoma or small neoplastic B cells). Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders.
  When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.
- No new sites of disease should be observed.
- Typically, FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET should be positive in at least one previously involved site. Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, CT

criteria should be used.

Stable disease is defined as less than a PR (see above) but is not progressive disease (see below):

- A patient is considered to have SD when he or she fails to attain the criteria needed for a CR or PR, but does not fulfill those for progressive disease (see Relapsed Disease [after CR]/Progressive Disease [after PR, SD]).
- Typically, FGD-avid lymphomas: the PET should be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET.
- Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan or if the pretreatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

Relapsed disease (after CR)/Progressive disease (after PR/SD) requires the following:

- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.
- At least a 50% increase from nadir in the SPD of any previously involved nodes, or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by ≥50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.
- At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
- Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (< 1.5 cm in its long axis by CT).

6.2.4 Time to Progression

Time to progression will be measured from the date of protocol consent until death of progressive disease is documented.

6.2.5 Immunologic Response Criteria

6.2.5.1 Definition of Biologically Active Dose

- ≥ 50% increase of the circulating absolute NK-cell number (product of absolute number of mononuclear cells and the proportion of NK cells) that is maintained for at least 1 week as determined by flow cytometry over the patient's pre-treatment NK-cell count (**APPENDIX B**). The baseline will be defined in each patient as the number of NK cells determined no more than 1 month prior to rhIL-15 administration.
- The study will aim at determining a range of biologically active doses meeting the above criteria.

6.2.5.2 Secondary Biologic Study Endpoints

In mice and non-human primates, another sensitive indicator of biological activity following IL-15 administration has been the expansion of the T-memory phenotype $CD44^{hi}CD8^{+}$ (T-cell memory phenotype compartment in mice) cell. Therefore, the secondary biological endpoint will aim at determining signs of expansion of the counterpart T-cell population in humans.

- Doubling in the absolute number of $CD45RO^{+}$ $CD8^{+}$ T-cells that is maintained for at least 1 week as determined by flow cytometry over the patient's pre-treatment$CD45RO^{+}$ $CD8^{+}$ T-cell count. The baseline will be defined in each patient as the number of these cells determined no more than 1 month prior to rhIL-15 administration.
- The immunogenicity of the infused rhIL-15 will be defined using a 2-arm capture ELISA method (**APPENDIX B**).
- At least a 2-fold increase in serum soluble IL-2R-alpha (sIL-2R-alpha) levels.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means 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' means 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 adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.
"Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience 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.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**

- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND Sponsor Reporting Criteria

During the first 30 days after the subject receives investigational agent/intervention, the investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only report those that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events including deaths due to progressive disease must be reported within one business day

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of

exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an AE unless there is a suspicion that the study treatment under study may have interfered with the effectiveness of a contraceptive medication. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as **Grade 3** "***Pregnancy, puerperium and perinatal conditions - Other (pregnancy)***" under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of rhIL-15.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 4 months after the last dose should, if possible, be followed up and documented.

7.4 DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and if applicable to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

8 STATISTICAL CONSIDERATIONS

The primary objective of this trial is to determine the safety, toxicity profile, DLT and MTD of CIV rhIL-15 administered for 10 consecutive days (240 hours) or 5 consecutive days (120 hours) in subjects with metastatic unresectable cancers for which curative or palliative measures either do not exist or are not associated with a survival advantage.

The secondary objectives include determination of rhIL-15 PK, including time to reach serum steady state, decline if any at later time points, changes in serum concentration associated with the expected lymphocytosis and decline following discontinuation of the CIV infusion; characterization of the biological effects of rhIL-15 on the percentages and absolute numbers of circulating lymphocyte sets (NK and T-cells) and T-cell subsets (naïve, central or effector memory subsets based on expression of CD56, CD4, CD8, CD45RO, CD45RA, CD28, CD95, CCR7 and CD62L) by flow cytometry and the plasma levels of pro-inflammatory cytokines. The potential antitumor activity of rhIL-15 will be assessed by the clinical response rate and time to progression in this patient population; and assessment of the nature of T-cell infiltration and cytokine and check-point inhibitor gene expression by analysis of pre- and post-treatment biopsies obtained from selected patients with easily accessible tumor deposits. All evaluations of these secondary endpoints will be considered exploratory.

There are 9 planned dose levels for this trial that will enroll 3 to 6 patients each using a modified Fibonacci design to define the MTD for CIV rhIL-15.

The MTD for each schedule will be based on the assessment of DLT during the first cycle of treatment, and will be defined as the dose level at which less than one-third of patients (0/3 or 0-1/6 patients) treated at that dose experience a DLT, with the next higher dose level demonstrating a one-third or greater number of patients ($\geq$ 2/3 or $\geq$ 2/6 patients) having DLT. If a patient did not experience DLT and did not finish 1 cycle of treatment (either 42 or 21 days), he or she will not be evaluable for determination of the MTD and will be replaced in the dose level. An additional 3 to 6 patients will be enrolled at the MTD or, if no severe or dose-limiting toxicities are encountered, the maximum administered dose of rhIL-15, so that a total of 9 patients will potentially be treated at MTD for either schedule to better characterize the clinical activity of

CIV rhIL-15 in this patient population. If feasible, the patients enrolled in this expansion cohort will be limited to histologic cancer subtypes previously shown to be sensitive to immunotherapy (renal cell carcinoma, melanoma, prostate, colorectal and ovarian cancer).

As of April 2016, twenty-four evaluable patients have enrolled on Dose Levels 1-6; with 2 more to be enrolled in Dose Level 6 after Amendment G. A maximum of 6 evaluable patients may be enrolled at DL 7 & 8 with an additional 9 at DL 9. If all dose levels are evaluated (with 6 patients per remaining dose levels and 9 total patients at the MTD or maximum administered dose), a maximum of 47 evaluable patients will be enrolled. An additional 5 patients may be enrolled to compensate for inevaluable patients; thus, bringing the maximum accrual ceiling to 52. Similarly, if all dose levels are evaluated, the minimum number of patients required will be 41 (24 evaluable patients have enrolled at DL 1-6; with 2 more to be enrolled in Dose Level 6 after Amendment G and with 3 patients enrolled at DL 7 & 8; 9 at DL 9; and 5 inevaluable patients a minimum of 46 will be reached).

Patients with tumors known to have tumor markers (PSA, CEA, CA125) will have the serum level of these markers assessed at the time of their radiographic restaging to gain further insight into the potential efficacy of rhIL-15 treatment. If tumor markers are initially above the upper limit of normal, they must normalize for the patient to be considered a complete response. Specific guidelines for serum tumor markers previously reported (CA125 and PSA) will be used to assess responses to rhIL-15 treatment.

Using the two dose escalation schedules, the probability of escalating to the next dose level, based on the true rate of DLT at the current dose for each schedule, is given by the following table (each group will be considered independently of the other):

True Toxicity at a Given Dose	10%	20%	30%	40%	50%	60%
Probability of Escalating	.91	.71	.49	.31	.17	.08

Thus, if the true underlying proportion of DLTs is 50% at the current dose, there is a 17% chance of escalating to the next dose.

9 COLLABORATIVE AGREEMENTS

9.1 MATERIAL TRANSFER AGREEMENT (MTA)

There is an NCI Intramural MTA # 14-2-00040 in place between DCTD, CTEP and CCR, NCI.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

Men and women age 18 years or older of all races and ethnic groups are eligible for this trial if they meet the criteria outlined. Efforts will be made to extend the accrual to a representative population. However, in a Phase I trial with limited sample size, a balance must be struck between patient safety, considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity were noted, accrual may be expanded or additional studies may

be performed to investigate those differences more fully. Because there is no significant preclinical information regarding the risk to a fetus or newborn infant, pregnant or breastfeeding women will be excluded from participation in this trial. Since the CIV rhIL-15 treatment acts by stimulating the patient's immune system to attack their tumor, patients with HIV, hepatitis B or C that have defective immune systems or immune responses much less likely to have benefit from this immune based therapy are not eligible for this trial.

Adults who are cognitively impaired prior to study entry will not be eligible for the trial, because they cannot give informed consent. Some of the subjects could become cognitively impaired because of disease progression or other causes. Patients will be offered the opportunity to assign Durable Power of Attorney (DPA) prior to study entry.

10.2 Participation of Children

Individuals under the age of 18 years of age are not eligible to participate in this trial. The immune physiology of children and the potential toxicity in this population are felt to be sufficiently different from those of adult patients that a separate protocol, dedicated to pediatric patients, appears warranted. Therefore, children will be the object of a separate protocol once toxicity and a dose range with biological activity are defined in adults.

10.3 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally-impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **10.6**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 Evaluation of Benefits and Risks/Discomforts

The discomforts of a 10-day CIV of rhIL-15 are expected to be the same pain and discomfort from venipuncture for blood drawing. The primary risk to patients participating in this research study is from expected or unforeseen toxicity or biological effects of recombinant human IL-15 as an investigational agent. Patients will be carefully monitored for any adverse events as described in Section **3.5** and appropriate care provided.

The intent of the study is to evaluate the toxicity of rhIL-15 in this Phase I trial and to find a range of doses where it has an effect on humans (biological activity). Recently there has been new evidence to suggest that patients with solid tumors may respond to immunotherapeutics or that infiltration of their tumor deposits by activated effector T-cells is associated with an improved survival. These finding lend additional weight to the notion that the administration of

rhIL-15 might be associated with a partial or complete response of the patient's tumor more commonly associated with the immune-based treatment of metastatic melanoma and metastatic renal cell cancer.

10.5 Strategies and Procedures for Recruitment

Patient accrual will be facilitated by the large number of clinical investigators who refer patients to the NCI and CCR. Contact letters or emails will be sent to the regional University referral center's Medical Oncologists informing them of this trial. In addition, the information will be provided on the NCI Clinical Trials and ClinicalTrials.gov web pages.

10.6 Risks/Benefits Analysis

The success of this effort cannot be predicted at this time. Because all patients in this protocol have incurable metastatic cancer and limited life expectancies, the potential benefit is thought to outweigh the potential risks.

Given the prospect of direct benefit, this same risks/benefits analysis applies also to adults who may become unable to consent during participation.

10.7 Consent Process and Documentation

The investigational nature and the research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, potential benefits, time commitments and potential alternative therapies will be carefully explained to the patients during the informed consent process. A signed informed consent document will be obtained prior to entry onto the study by the Principal Investigator or an Associate Investigator. The original signed consent goes to Medical Records and a copy will be placed in the research record.

If the patient agrees to have the optional biopsy for research, then the patient will consent at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

10.7.1 Telephone reconsent

Reconsent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the change (s) in the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

10.7.2 Informed consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2) and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed

copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation. Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

11.1 RECOMBINANT HUMAN INTERLEUKIN-15

Chemical Name: Recombinant human interleukin-15 (rhIL-15)

Other Names: Interleukin-T (IL-T)—no longer recognized.

Classification: Recombinant human cytokine.

Molecular Structure: Member of the four α-helix bundle family of human cytokines consisting of 115 amino acids manufactured in *E. coli* by recombinant technology. Non-glycosylated.

Description: Sterile, clear, colorless, particulate-free liquid; endotoxin free.

11.1.1 Source

Agent Ordering and Agent Accountability
NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent

management.

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: PMBRegPend@ctep.nci.nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx
- CTEP Identity and Access Management (IAM) account: https://eapps-ctep.nci.nih.gov/iam/
- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

11.1.2 Toxicity

From the experience in the ongoing rhIL-15 IVB trial, side effects of fatigue, fever, rigors or chills, capillary leak syndrome, fluid retention and edema are likely. Although there has not been any evidence of meaningful pulmonary dysfunction or changes in renal function, the preclinical NHP data and experience with other similar biological compounds indicates that these toxicities could potentially occur in patients treated with the CIV schedule. Gastrointestinal toxicities in the IVB IL-15 patients have included nausea, vomiting and in one case explosive diarrhea exclusively in the patient treated with the 3 mcg/kg/day dose, and data from the NHP toxicology study also showed diarrhea and dehydration—these could also be more common in patients treated with the CIV regimen. Although there has not been significant granulocytopenia in the rhIL-15 patients treated to date and lymphopenia has been transient, there is still some potential for infectious issues if the granulocytopenia associated with CIV treatment of macaques occurs in the patients enrolled in this protocol.

Comprehensive Adverse Events and Potential Risks List (CAEPRs) For Recombinant Human IL-15

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system.

Version 1.2, February 01, 2016[1]

Adverse Events with Possible Relationship to Recombinant Human IL-15 (CTCAE 4.0 Term)	Specific Protocol Exceptions to Expedited Reporting (SPEER)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	
Anemia	***Anemia (Gr 2)***
Bone marrow hypocellular	
CARDIAC DISORDERS	
Sinus tachycardia	***Sinus tachycardia (Gr 2)***
GASTROINTESTINAL DISORDERS	
Abdominal pain	
Diarrhea	
Nausea	***Nausea (Gr 2)***
Vomiting	***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	
Chills	***Chills (Gr 2)***
Edema limbs	
Fatigue	***Fatigue (Gr 2)***
Fever	***Fever (Gr 2)***
Injection site reaction	
INFECTIONS AND INFESTATIONS	
Sepsis	
INVESTIGATIONS	
Alanine aminotransferase increased	
Aspartate aminotransferase increased	
Blood bilirubin increased	
Creatinine increased	
Lymphocyte count decreased	***Lymphocyte count decreased (Gr 2)***
Lymphocyte count increased	
Neutrophil count decreased	
Platelet count decreased	
White blood cell decreased	
METABOLISM AND NUTRITION DISORDERS	
Hypoalbuminemia	
Hypophosphatemia	***Hypophosphatemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	
Generalized muscle weakness	
NERVOUS SYSTEM DISORDERS	
Dizziness	
Headache	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	
Dyspnea	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	
Dry skin	
Erythema multiforme	***Erythema multiforme (Gr 2)***
Skin and subcutaneous tissue disorders - Other (rash)	
VASCULAR DISORDERS	
Capillary leak syndrome	
Hypertension	***Hypertension (Gr 2)***
Hypotension	***Hypotension (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised.

Adverse events reported on Recombinant Human IL-15 trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Recombinant Human IL-15 caused the adverse event:

Blood and lymphatic system disorders - Febrile neutropenia

Cardiac disorders - Atrial fibrillation; Chest pain - cardiac; Palpitations; Pericardial effusion; Pericardial tamponade; Sinus bradycardia; Ventricular tachycardia

Gastrointestinal disorders - Ascites; Constipation; Duodenal hemorrhage; Gastritis; Gastrointestinal disorders - Other (increased appetite); Ileus; Mucositis oral; Pancreatitis

General disorders and administration site conditions - Edema face; Infusion related reaction; Infusion site extravasation; Multi-organ failure; Pain

Immune system disorders - Autoimmune disorder

Infections and infestations - Tooth infection; Upper respiratory infection; Urinary tract infection

Injury, poisoning and procedural complications - Bruising

Investigations - Alkaline phosphatase increased; Cardiac troponin I increased; Electrocardiogram QT corrected interval prolonged; GGT increased; INR increased; Lipase increased; Serum amylase increased; Weight gain; Weight loss

Metabolism and nutrition disorders - Anorexia; Dehydration; Hyperkalemia; Hypocalcemia; Hypokalemia

Musculoskeletal and connective tissue disorders - Back pain; Bone pain; Muscle weakness upper limb; Myalgia; Pain in extremity

Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Tumor pain

Nervous system disorders - Dysgeusia; Peripheral sensory neuropathy; Presyncope; Vasovagal reaction

Psychiatric disorders - Anxiety; Psychosis

Reproductive system and breast disorders - Genital edema

Respiratory, thoracic and mediastinal disorders - Adult respiratory distress syndrome; Bronchopulmonary hemorrhage; Cough; Hypoxia; Laryngeal inflammation; Pleural effusion; Pneumonitis; Pulmonary edema; Wheezing

Skin and subcutaneous tissue disorders - Erythroderma; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Rash acneiform; Skin and subcutaneous tissue disorders - Other (skin plaques)

Vascular disorders - Hot flashes; Visceral arterial ischemia

Note: Recombinant Human IL-15 in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

11.1.3 Formulation

Sterile rhIL-15 drug product is vialed at a concentration of 0.52 mg/mL (0.16 mg/0.3 ml) solution of 25 mM sodium phosphate, dibasic and 500 mM sodium chloride (pH 7.0 ±0.4) in a 5 mL borosilicate glass vial. rhIL-15 drug product for intravenous administration should be

withdrawn from the vial and prepared as described in Section **11.1.5**.

Vials are for single use only.

11.1.4 Stability and Storage
Long-term at ≤ -70ºC.

Storage of rhIL-15 diluted in 0.1% HSA in infusion bags is limited to a maximum of 4 hours at room temperature or 24 hours at 2-8°C prior to initiation of the 24- hour infusion.

Drug stability studies are ongoing and will be continued throughout the clinical trial. Long-term drug stability information will be periodically updated and communicated to the Clinical Center Pharmacy Department.

11.1.5 Preparation and Administration Procedures
For all dose levels, the dose of rhIL-15 will be diluted in the appropriate volume of 0.1% human serum albumin (HSA) in 5% dextrose in water, USP (D5W) to reach a final rhIL-15 concentration of 1 mcg/mL. The rhIL-15 infusion will be administered to the patient by continuous intravenous infusion (CIV) over 24 hours using a portable ambulatory pump on the inpatient unit for either a total of 240 or 120 hours depending on the treatment cohort. Treatment with rhIL-15 will begin within 4 hours of preparation of the infusion bag and the infusion must be completed within 24 hours from the time drug administration begins.Otherwise a new infusion bag must be prepared to complete administration of the remaining dose.

The infusion solutions will be mixed in an MMI bag.

The rhIL-15 infusion solution is stable at a concentration of 1.0 mcg/mL with 0.1 HSA for 4 hours at controlled room temperature (15°C–30°C) prior to initiation of the 24-hour infusion or 24 hours at 2-8ºC prior to initiation of the 24-hour infusion. This stability information was previously documented by the Biopharmaceutical Development Program (BDP) of Leidos Biomedical Research, Inc., the drug manufacturer.

11.1.6 Drug Incompatibilities
None known.

12 REFERENCES

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13 APPENDICES

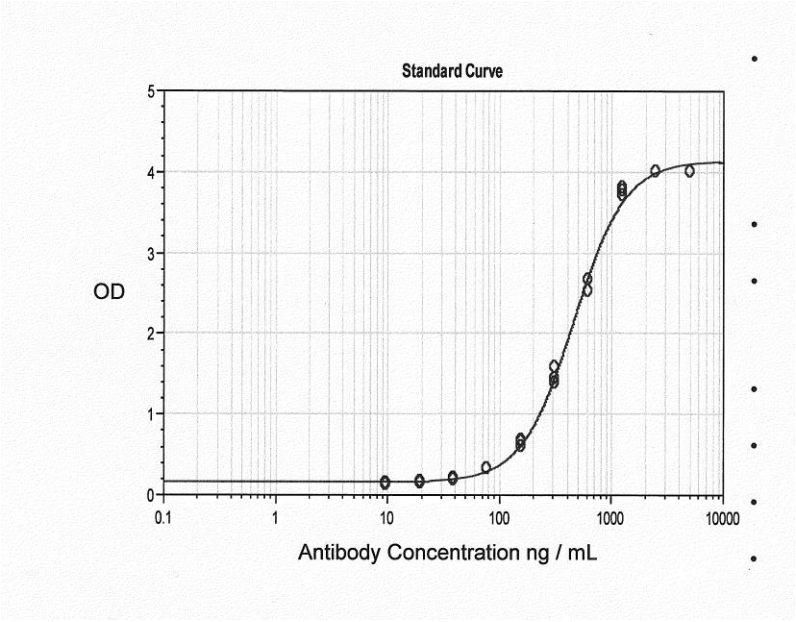
APPENDIX A: PERFORMANCE STATUS CRITERIA

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

APPENDIX B: IMMUNE-BASED STUDIES

1. Flow Cytometry
    - Flow cytometry analysis for peripheral blood lymphocyte subset enumeration will be performed using standard techniques in the College of American pathologists (CAP) certified flow cytometry laboratory within the Clinical Pathology Department, NIH Clinical Center.
    - Special flow cytometry also performed by Dr. Enrico Lugli with Dr. Mario Roederer in the Vaccine Research Center, Building 40, Room 5044.
2. Assay of rhIL-15 by R&D Systems Quantikine ELISA Kit (Catalog # D1500) to define concentration of IL-15 in pharmacokinetic assay.
3. Assay for antibodies to infused IL-15 (see **APPENDIX C**).

APPENDIX C: ASSAY FOR ANTIBODIES TO RHIL-15



- Plates are coated with human IL-15 for 3 hours at 37°C, washed, blocked with 3% FBS and washed again.
- A standard curve for assay quantitation and quality control is constructed using serial dilutions of a commercial affinity purified goat anti-human IL-15 that is diluted in heat-inactivated normal human serum. The standard curve samples are incubated for 2 hours at 37°C and washed.
- Biotin conjugated IL-15 is added to each well, incubated 2 hours at 37°C, and the plates are washed.
- Alkaline phosphatase–conjugated streptavidin is added to each well for 2 hours at 37°C and then washed.
- The assay is developed with the addition of diethanolamine buffer with p-Nitrophenyl Phosphatase for 1 hour at 37°C and then immediately read at 405 nm.
- To detect antibodies to human IL-15 in test samples, serum from the test subject will be assayed in duplicate at dilutions of 1/3 and 1/9 concomitantly with the standard curve samples as above, and the resultant OD obtained used to quantitate the level of antibody present.

APPENDIX D: CIV RHIL-15 BIOSPECIMEN WORKSHEET FOR 12-C-0113 CYCLE 1 FOR 10 DAY SCHEDULE

Work sheet for 12-C-0113 Cycle 1

Patient Name:	Start Date of Treatment: __/__/20 _____ Start Time of Treatment: ____	Dose Level: _____mcg/kg/day

All samples should be kept at Room Temperature in Clean Utility Room

Page Figg Lab (pager 102-11964) for Sample pick up | **Page LyMB research nurse (pager 102-11029) for sample pick up**

Study Day	Sample name	Specimen Information Tube		Sample type	Ideal Sample Time	Actual Time Drawn	Pick up	Comments (Missed Specimens, etc.)	Signature
1	IL-15 antibodies	1	4 ml SST	ELISA			Figg		
	IL-15 Level PreRx	1	8 ml SST	PK					
	Cytokines MesoScale Pre Rx			ELISA					
	IL-15 level 10 min	1	4 ml SST	PK					
	IL-15 level 1hr	1	4 ml SST	PK					
	IL-15 level 2hr	1	4 ml SST	PK					
	IL-15 level 4hr	1	8 ml SST	PK					
	Cytokines MesoScale 4 hr.			ELISA					
	IL-15 level 8hr	1	8 ml SST	PK					
	Cytokines MesoScale 8 hr.			ELISA					
	IL-15 level 12hr	1	4 ml SST	PK					
2	IL-15 level 24hr	1	8 ml SST	PK					
	Cytokines MesoScale 24 hr.			ELISA					
	Roederer FACS day 2	2	10 ml Lavender	Immune subsets			LyMB		
3	IL-15 level 48hr	1	4 ml SST	PK			Figg		
7	IL-15 level day 7	1	4 ml SST	PK	Draw with AM Labs		Figg		

1

Patient Name:	Start Date of Treatment: __/__/20_ Start Time of Treatment: _____	Dose Level: _____ mcg/kg/day

All samples should be kept at Room Temperature in Clean Utility Room Page until Pick up

Page Figg Lab (pager 102-11964) for Sample pick up | **Page LyMB research nurse (pager 102-11029) for sample pick up**

Study Day	Sample name	Specimen Information: Tube		Sample Type	Ideal Sample Time	Actual Time Drawn	Pick up	Comments (Missed Specimens, etc.)	Signature
8	IL-15 level day 8	1	8 ml SST	PK	Draw with AM Labs		Figg		
	Cytokines MesoScale day 8			ELISA					
	Roederer FACS day 8	2	10 ml Lavender	Immune subsets	Draw with AM Labs		LyMB		
9	IL-15 level day 9	1	4 ml SST	PK	Draw with AM Labs		Figg		
10	IL-15 level day 10	1	4 ml SST	PK	Draw with AM Labs				
11	IL-15 Level End of Rx	1	4 ml SST	PK					
	IL-15 Level 10 min post Rx	1	4 ml SST	PK					
	IL-15 Level 30 min post Rx	1	4 ml SST	PK					
	IL-15 Level 1 hr. post Rx	1	4 ml SST	PK					
	IL-15 Level 2 hr. post Rx	1	4 ml SST	PK					
	IL-15 Level 4 hr. post Rx	1	4 ml SST	PK					
12	IL-15 Level 24 hr. post Rx	1	8 ml SST	PK					
	Cytokines MesoScale day 12			ELISA					
	Roederer FACS day 12	2	10 ml Lavender	Immune subsets	Draw with AM Labs		LyMB		
	IL-15 antibodies	1	4 ml SST	ELISA	Draw with AM Labs		Figg		

APPENDIX E: CIV RHIL-15 BIOSPECIMEN WORKSHEET FOR 12-C-0113 CYCLE 2 AND BEYOND

Work sheet for 12-C-0113 Cycle 2

Patient Name:	**Start Date of Treatment: __/__/20__** **Start Time of Treatment: _____**	**Dose Level: _____mcg/kg/day**

All samples should be kept at Room Temperature in Clean Utility Room Page until Pick up	
Page Figg Lab (pager 102-11964) for Sample pick up	**Page LyMB research nurse (pager 102-11029) for sample pick up**

Study day	**Sample name**	**Specimen Information**			**Ideal Sample Time**	**Actual Time Drawn**	**Pick up**	**Comments (Missed Specimens, etc.)**	**Signature**
		Tube		**Sample type**					
1	**IL-15 antibodies**	**1**	**4 ml SST**	ELISA	**Draw with AM Labs**		**Figg**		
2	**Roederer FACS day 2**	**2**	**10 ml Lavender**	Immune subsets	**Draw with AM Labs**		**LyMB**		
8	**Roederer FACS day 8**	**2**	**10 ml Lavender**	Immune subsets	**Draw with AM Labs**				
12	**Roederer FACS day 12**	**2**	**10 ml Lavender**	Immune subsets	**Draw with AM Labs**				
	IL-15 antibodies	**1**	**4 ml SST**	ELISA	**Draw with AM Labs**		**Figg**		