Abbreviated Title: SC IL-15 + alemtuzumab in ATL
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A Phase I Study of Subcutaneous Recombinant Human IL-15 (S.C. Rhil-15) and Alemtuzumab for Patients with Refractory or Relapsed Chronic and Acute Adult T-Cell Leukemia (ATL)

NCI Principal Investigator: Kevin Conlon, M.D.
Center for Cancer Research (CCR)
National Cancer Institute
Building 10, Room 3B38
9000 Rockville Pike
Bethesda, MD 20892
Phone: 240-760-6087
Email: conlonkc@mail.nih.gov

Investigational Agent:

Drug Name:	Recombinant human IL-15 (rhIL-15) (NSC #745101)	Alemtuzumab
IND Number:	129263	
Sponsor:	Center for Cancer Research (CCR), NCI	
Manufacturer:	Biopharmaceutical Development Program (BDP)/Leidos Biomedical Research, Inc. under contract with Division of Cancer Treatment and Diagnosis (DCTD), NCI (CTEP Protocol #10004)	Genzyme Corporation
Supplier:	BDP/DCTD, NCI	NIH Pharmacy

PRÉCIS

Background:

- A previous trial of alemtuzumab (CAMPATH-1) in patients with chronic, acute and lymphomatous subtype HTLV-1 associated ATL showed appreciable initial activity but no clear long-term impact.

  Antibody dependent cellular cytotoxicity (ADCC) with polymorphonuclear neutrophils (PMNs), monocytes and natural killer (NK) cells acting as the effector cells is alemtuzumab's primary *in vivo* mechanism of action for depleting malignant leukemic or lymphomatous cells.
- The immunologic effects of Interleukin-15 (IL-15), a stimulatory cytokine that promotes the differentiation and activation of NK cells, monocytes and long-term $CD8^+$ memory T-cells, has been assessed in several phase I trials in cancer patients.
- Administration of recombinant human (rh) IL-15 as an intravenous bolus (IVB), continuous intravenous infusion (CIV) or subcutaneous injections (SC) into adult cancer patients has produced 5- to 50-fold expansion in the number of circulating NK cells at well-tolerated doses in these patients.
- Preclinical murine lymphoid malignancy models have shown efficacy from the administration of IL-15 and monoclonal antibodies, with improved survival compared to controls.

Objective:

- To determine the safety, toxicity profile and the maximum tolerated dose (MTD) of s.c. rhIL-15 in combination with standard three times per week IV alemtuzumab treatment

Eligibility:

- Age ≥ 18-years old
- ECOG Performance Status ≤ 1
- Diagnosis of adult T-cell leukemia (HTLV-1 associated, chronic or acute), peripheral T-cell lymphoma (angioimmunoblastic, hepatosplenic, or not otherwise specified), cutaneous T-cell lymphoma (Stage III or IV, with leukemic involvement or erythrodermia), or T-cell prolymphocytic leukemia (T-PLL)
- Measurable or evaluable disease
- Adequate organ and bone marrow function as defined in the protocol.

Design:

- This is a single institution nonrandomized Phase I dose escalation study evaluating increasing doses of SC rhIL-15 in combination with alemtuzumab using a standard 3 + 3 dose escalation.
- Treatment will include s.c. rhIL015 daily (M-F) weeks 1 and 2 (dose levels 0.5-2mcg/kg/dose), followed by IV alemtuzumab beginning in week 3 (escalating doses followed by standard dosing in weeks 4-6).

  Up to 30 patients will be enrolled in this study.

SCHEMA

Cohort number	rhIL-15 Dose	Treatment	Weeks					
			1	2	3	4	5	6
Cohort 1	0.5mcg/kg/dose	rhIL-15	→	→				
		alemtuzumab			↓↓↓	→	→	→
Cohort 2	1 mcg/kg/dose	rhIL-15	→	→				
		alemtuzumab			↓↓↓	→	→	→
Cohort 3	2 mcg/kg/dose	rhIL-15	→	→				
		alemtuzumab			↓↓↓	→	→	→

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Determine the safety, toxicity profile and the maximum tolerated dose (MTD) of s.c. rhIL-15 administered in combination with standard three times per week IV alemtuzumab treatment.

1.1.2 Secondary Objectives

- Evaluate the potential antitumor activity of the combination of rhIL-15 and alemtuzumab assessing the clinical response rate and progression free survival.
- Characterize the biological effects of rhIL-15 administered with alemtuzumab on the percentages and absolute numbers of circulating lymphocytes (T and NK cells) and the T-cell subsets naïve, central and effector memory subsets (based on expression of CD52, CD56, CD4, CD8, CD45RO, CD45RA, CD28, CD95, CD194, CCR7 and CD62L) by flow cytometry.
- Define the effect of the combination on the plasma levels of pro-inflammatory cytokines.

1.2 BACKGROUND AND RATIONALE

1.2.1 Adult T-cell leukemia (ATL)

Epidemiological and clinical features of HTLV-1 associated ATL:

ATL is an aggressive T-cell lymphoproliferative disorder characterized by the presence of malignant CD4/CD25 expressing T-cells in the peripheral blood and in lymphoid and other tissues(1-3). The disease exhibits a striking clustering of cases in certain geographic regions, notably southwestern Japan, the Caribbean basin, northeastern South America, Central Africa and the southeastern United States. Epidemiologic studies demonstrate a clear association of the disease with the presence of a retrovirus human T-cell lymphotropic virus, type 1 (HTLV-1)(4-

6). The vast majority of individuals with HTLV-1 infection are asymptomatic carriers with an approximately 0.1 percent per year risk of developing frank ATL leading to 2 to 5 percent cumulative risk of developing this malignancy. Most often ATL has its onset 20-30 years following perinatal infection from the mother infected with HTLV-1(4).

ATL cells exhibit characteristic morphological features (flower-like cells) with deeply indented nuclei, derived from mature T-reg cells with the surface phenotype ($CD3^+$, $CD4^+$ $CD7^-$, $CD8^-$ $CD25^+$)(1, 4). While the overall genomic structure of HTLV-1 is similar to that of other retroviruses(6), the presence of a region called pX, which encodes nonbearing proteins including 42-kDa Tax and 27-kDa Rex, is a relatively novel feature of the HTLV-1 genome(6). Tax is a transactivating transcription factor that activates the HTLV-1 long terminal repeat (LTR) leading to the expression of viral genes. The Tax protein induces the transcription of a number of immunologic cellular genes that include Interleukin 2 receptor alpha (IL-2Rα), IL-2, IL-3, IL-9, IL-13, IL-15, IL-15Rα, tumor necrosis factor (TNF), granulocyte-macrophage colony stimulating factor (GM-CSF), transforming growth factor beta-1 (TGFβ-1), parathyroid hormone related protein (PTHrP), vimentin and c-fos(4). All ATL cells express HBZ from the minus strand of HTLV-1 provirus. Previous work in the Waldmann lab has shown that HTLV-1 infection of T cells *in vivo* and *in vitro* lead to the constitutive expression of two autocrine loops (IL-2/IL-2R alpha, IL-15/IL-15Rα) and one paracrine loop (IL-9/ IL-9Rα) thereby leading to T-lymphocyte immortalization(7). The data indicate these autocrine and paracrine loops are largely a feature of the smoldering and chronic subtypes and that the IL-2 loop is probably more critically involved as indicated by the impact of anti-IL-2 or anti-IL-15 antibody treatment of the cellular cultures(7). It has been proposed that in the early phases (smoldering and chronic) of ATL the HLTV-1 induced leukemogenesis may be the result of Tax expression through its stimulatory effects on genes involved in cellular proliferation.

The clinical aggressiveness of ATL varies from patient to patient with a very broad spectrum. ATL has been subdivided into four categories(8): (1) smoldering type whose characteristics are 5 percent or more abnormal T lymphocytes in the peripheral blood in association with a normal lymphocyte level ($< 4 \times 10^9$/L), lack of hypercalcemia, lactic dehydrogenase (LDH) values no greater than 1.5 x the normal upper limit and no lymphadenopathy or organ involvement other than skin and pulmonary lesions. Patients with ATL demonstrable on skin biopsy do not have to manifest 5 percent abnormal cells in peripheral blood; (2) chronic type, absolute lymphocytosis (4×10^9/L or more) with T-cell lymphocytosis more than 3.5×10^9/L, LDH values up to twice the upper limit of normal and no hypercalcemia or involvement of the central nervous system, bone or gastrointestinal tract or manifestations of associated ascites or pulmonary effusions; (3) lymphoma type, no lymphocytosis, 1 percent or less abnormal T cells in the circulation, in conjunction with histologically proven malignant lymphadenopathy; and (4) acute type, that includes the remaining ATL patients who usually have leukemic manifestations and tumor lesions.

1.2.2 Treatment of ATL

1.2.2.1 Chemotherapy for ATL

Currently there is no curative therapeutic option for patients with HTLV-1 associated ATL outside of an allogeneic stem cell transplant (alloSCT). Experience with the standard combination chemotherapy regimens known to be useful in the treatment of the more common aggressive B-cell non-Hodgkin's lymphomas or acute lymphoblastic leukemia has been very disappointing in ATL(9, 10). The majority of patients with the three more aggressive categories

of ATL—severe chronic, acute and lymphoma type, have been treated with combination therapy including CHOP, (cyclophosphamide, Adriamycin, vincristine and prednisone), VEPA (vinblastine, etoposide, prednisone, and adriamycin), a Japanese Lymphoma Study Group protocol combining nine drugs (doxorubicin, cyclophosphamide, vincristine, prednisone, vindesine, methotrexate, etoposide, procarbazine, and bleomycin) or a comparable protocol with these drugs plus cisplatin(10). A total of 854 patients with HTLV-1 antibody positive ATL newly diagnosed from 1983-1987 were analyzed for prognostic factors and survival following combined chemotherapy by the Japanese Lymphoma Study Group(9). The median survival time (MST) and projected 2-year and 4-year survival rates of all patients were 10 months 28 percent, and 12 percent respectively. Impaired performance status, high lactic dehydrogenase values, age of 40 years or more, increased number of lesions and hypercalcemia were associated with shortened survival. Survival data have been analyzed according to clinical subtype, with most cases receiving combination chemotherapy. MST was 6.2 months for acute type, 10.2 months for lymphoma type and 24.3 months for chronic type. Projected 4-year survival rates were 5 percent for acute type, 5.7 percent for lymphoma type, 26.9 percent for chronic type and 58 percent for smoldering type. The members of the Lymphoma Study Group after reviewing 854 cases of ATL under various treatments concluded: “The various combination chemotherapies so far developed have not increased significantly the survival of patients with ATL”(9).

Partial (6) or complete (5) remissions were observed in 11 of 19 patients with ATL treated with a combination of interferon alpha and AZT(11), but the median survival for all patients was only 3 months, and 13 months for patients with complete or partial responses. A Meta-analysis suggested that AZT therapy in combination with interferon may provide value however it has shown lack of efficacy in patients with prior chemotherapy that have p53 mutations(12).

1.2.2.2 Therapy of ATL with Receptor-Directed Monoclonal Antibodies

The Waldmann Lab has utilized xenograft murine models of human ATL to evaluate therapeutic non-chemotherapy strategies that involve a monoclonal antibody directed toward receptors expressed on the surface of ATL cells. Using the MET-1 *in vivo* model of ATL, we demonstrated efficacy with the anti-CD2 monoclonal antibody MEDI-507 (Siplizumab)(13), the anti-CD52 monoclonal antibody, alemtuzumab (CAMPATH-1)(14) and the anti-CD25 antibody daclizumab(15)in this preclinical model system. A similar study with alemtuzumab in a severe combined immunodeficiency (SCID) model of NHL reported that treatment with alemtuzumab effectively inhibited growth of systemically disseminated malignant lymphoma cells and prolonged survival(16). On the basis of encouraging preclinical results, Siplizumab, alemtuzumab and daclizumab have been translated into clinical trials involving patients with T-cell malignancies with partial and complete responses observed(17).

1.2.2.3 Alemtuzumab (CAMPATH-1H) for ATL

Alemtuzumab is a humanized antilymphocyte monoclonal antibody targeting CD52, which was engineered by grafting the 6 rodent hypervariable complementarity-determining regions (CDR) from the heavy and light-chain variable domains of the rat monoclonal antibody (CAMPATH-1G) into a human immunoglobulin G1 kappa variable framework region(18). Alemtuzumab is directed at CD52, a 12 amino acid protein that is highly glycosylated and linked to the cell membrane by phosphatidylinositolglycan linkage. Alemtuzumab is thought to mediate cell lysis through complement and antibody-dependent, cell-mediated cytotoxicity. CD52 is expressed on monocytes but monocytes appear resistant to alemtuzumab mediated lysis. The expression of CD52 varies within the different sets and subsets of human lymphocytes with NK cells showing

the lowest expression and generally being more resistant to alemtuzumab mediated depletion(19, 20), leaving many mononuclear cells with the capacity for ADCC (**Figure 1**).

A key supposition for this trial is that CAMPATH-1 will not unduly deplete Fc-gamma receptor expressing IL-15 dependent immune subsets that also co-express the surface marker CD52. In support of this assumption, as just noted, NK cells show the lowest expression of CD52 and monocytes appear resistant to alemtuzumab refractory to CAMPATH-1 mediated complement lysis(20) (**Figure 2**). Previously we demonstrated that CAMPATH-1 provided effective therapy for a murine model of adult T-cell leukemia in wild-type mice but that all efficacy was lost in FcR gamma $^{-/-}$ mice supporting the view that ADCC is the dominant mechanism of efficacy in mice(14). The fact that alemtuzumab showed efficacy in the therapy of patients with acute ATL (12 of 17 patients manifesting a PR or CR) and chronic ATL (2 of 3 patients manifesting a response) supports our view that alemtuzumab will not unduly deplete Fc gamma receptor expressing immune subsets required for ADCC.

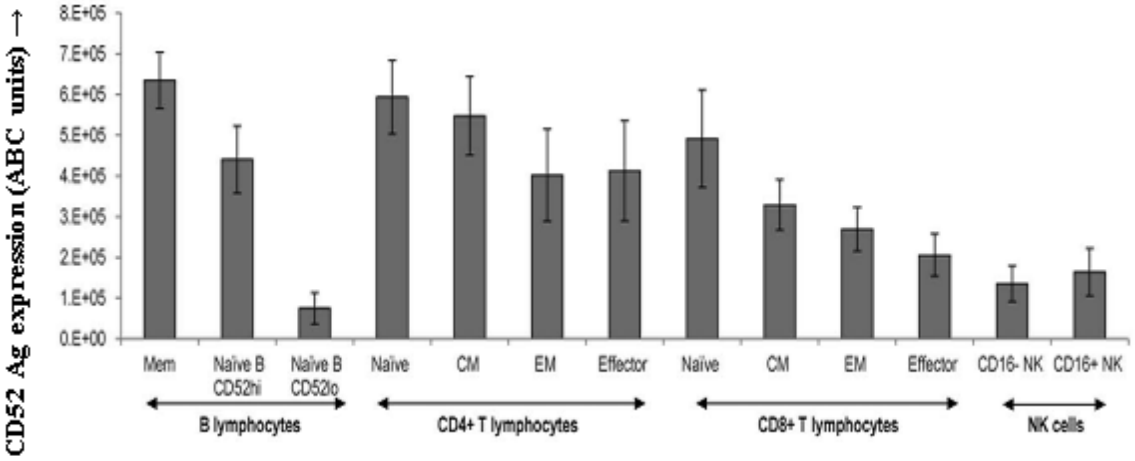

Figure 1: CD52 antigen density on Human PBMC subsets. CD52 expression was determined on subsets of freshly isolated PBMCs from 22 normal donors. [Adapted from Figure 4 Rao et al.(**20**)]

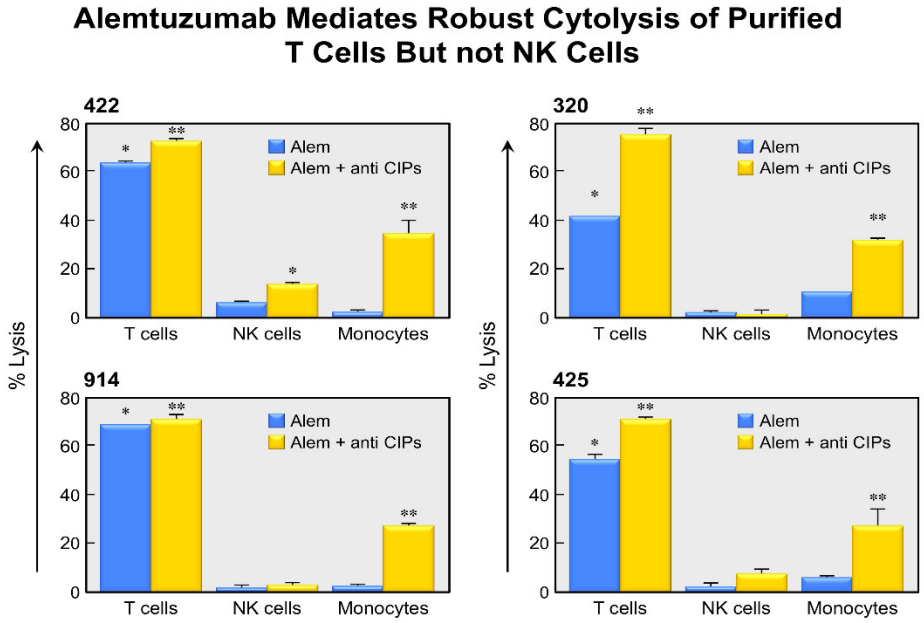

Figure 2: Alemtuzumab mediates robust cytolysis of purified T cells but not NK cells. Blocking anti-CIP antibodies partially reverse the resistance of monocytes to lysis by alemtuzumab. Adapted from Figure 8 Rao et al(**20**).

Alemtuzumab has demonstrated anticancer activity in patients with B-CLL(21-23), non-Hodgkin's lymphoma(24, 25), T-cell prolymphocyte leukemia(26-28), mycosis fungoides/Sézary syndrome(29-31). A very large number of patients have been studied with alemtuzumab by Wellcome, Inc. and an approach with 3 mg on day one, 10 mg on day two and 30 mg on day three, followed by 30 mg administered intravenously 3 x weekly for 12 weeks was selected for evaluation in patients with non-Hodgkin's lymphoma and chronic lymphocytic leukemia. The incidence of common adverse events seen in about half of the treated patients included chills/rigors, fever, nausea, vomiting and skin rash. Hypotension occurred in about a third of patients but did not achieve grade 3 or 4 levels, dyspnea appeared in about 25% of admissions, and bronchospasm occurred in about 10% of patients. Neutropenia emerged during treatment in about 24% of patients. Thrombocytopenia occurred in association with early infusions in the course of treatment and typically became less pronounced with continued treatment. There were 27 deaths among 174 patients within 28 days of the final alemtuzumab infusion. Fourteen deaths were attributed to disease progression, and 6 were due to infectious complications.

Tumor responses were observed in 16 patients, 8 occurred in patients with CLL or PLL leading to the approval of alemtuzumab for this indication. Alemtuzumab was granted initial approval by the U.S. Food & Drug Administration (U.S. FDA) as injection therapy for intravenous use indicated as a single agent for the treatment of B-cell chronic lymphocytic leukemia (B-CLL) in 2001. In 2007 the U.S. FDA granted approval for the use of alemtuzumab in previously untreated B-CLL patients after evaluation in an open-label randomized: (1.) active—controlled trial in previously untreated patients with B-CLL and (2.) Rai Stage I-IV, with evidence of progressive disease requiring therapy(32).

1.2.2.4 LYMB Experience with Alemtuzumab Treatment for ATL

Examination of leukemic cells in fine needle aspiration biopsies demonstrated that CD52 is highly expressed in over 90% of ATL cells and is comparable to the level of CD25 expression observed on the tumor cells in these patients(33). In preclinical studies alemtuzumab prolonged the survival of mice with human MET-1 ATL comparable to that of tumor-free controls(14). These results were the basis of a single institution, nonrandomized, open-label Phase II clinical trial which was initiated to assess the antitumor activity and toxicity profile of alemtuzumab in patients with HTLV-1 associated ATL, excluding those with the smoldering subtype(33). Twenty-nine patients with chronic, acute and lymphomatous HTLV-1 associated ATL were treated with intravenous alemtuzumab 30 mg three times weekly for a maximum of 12 weeks in this single institution, non-randomized, open-label Phase II trial. All patients were evaluable for AEs and response with the exception that one patient was not evaluable for response because of early withdrawal. The overall response rate was 15 out of 29 patients (95% CI: 32.5 to 70.6%), but response rate varied widely within the different ATL subtypes. Only 1 of 9 patients with lymphomatous subtype responded, in contrast to the acute subtypes (12 of 17) and 2 of 3 patients with severe chronic ATL. The most common adverse events were infusion reactions that were grade 1 or 2 (CTCAE v2.0) occurring mainly in the first week of alemtuzumab administration and the median time to recovery of ALC to ≥ 200 cells/μL was 1.8 months. All patients developed CMV antigenemia, but only 3 of the patients became symptomatic and required therapeutic antiviral treatment. In the 15 responding patients, the median time to response was 1.1 month (range 1.0 to 4.2 months), but the median response duration (3.4 months) and the median progression free survival (2.0 months) were short producing a short period of clinical benefit, median time to treatment failure (1.6 months) and median survival (5.9 months) with only 6 patients (21%) alive at the date of the data cutoff (November 2009) (**Figure 3**).

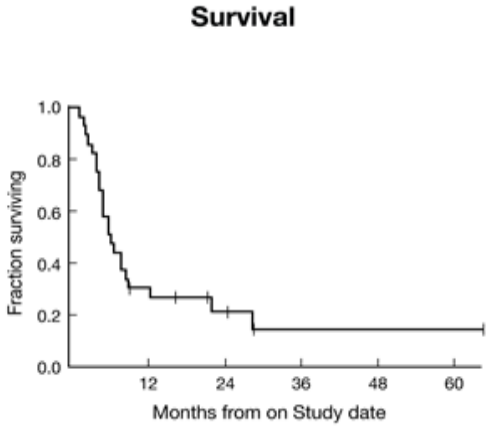

Figure 3: Meier-Kaplan Plot of Survival of patients with ATL (3 chronic, 17 acute and 9 lymphomatous) who were treated with alemtuzumab

Although alemtuzumab had a reasonable early response rate in ATL with acceptable level of toxicity, the duration of the response was short and there was not a meaningful clinical impact on the disease course. These results suggest that combining alemtuzumab with an agent that could potentiate the antibody's ADCC capacity may provide clinically important synergy.

1.2.3 Other $CD52^+$ mature T-cell malignancies

Mature T-cell malignancies have variable expression of CD52; this variability is further

confounded by different methods of assessment (Table 1). Overall, 40-100% of angioimmunoblastic T-cell lymphoma, 33-100% of hepatosplenic T-cell lymphoma, and 35-100% of peripheral T-cell lymphoma, not otherwise specified were $CD52^{+}$, with higher percentages reported in studies using the more sensitive immunofluorescence and flow cytometry(19, 34-36). Only 5-50% of anaplastic large cell lymphoma(19, 34) and 25-33% of extranodal T/NK-cell lymphoma(19, 36) showed CD52 expression, with higher expression reported in the smaller series. In the only report of CD52 expression in cutaneous T-cell lymphoma, 87.5% of samples were positive by flow cytometry(19).

Table 1: Reported expression of CD52 marker in mature T-cell malignancies; PTCL = peripheral T-cell lymphoma NOS, CTCL = Cutaneous T-cell lymphoma, ALCL = anaplastic large cell lymphoma, AITCL = angioimmunoblastic T-cell lymphoma, TNKCL = extranodal T/NK-cell lymphoma, HSTCL = hepatosplenic T-cell lymphoma, FC = flow cytometry, IF = immunofluorescence, IHC = immunohistochemistry, GEP = gene expression profiling

Study	Method	PTCL	CTCL	ALCL	AITCL	TNKCL	HSTCL
Jiang, 2009(19)	FC	12/13	14/16	1/2	3/3	1/4	4/4
Geissinger 2009(34)	IF	5/5		1/18	6/6		
Piccaluga, 2007(35)	IHC GEP	40/97 11/28					
Rodig, 2006(36)	IHC	7/20			2/5	1/3	2/6

1.2.3.1 Treatment of relapsed/refractory PTCL

Two agents, pralatrexate and belinostat, have received accelerated FDA approval for treatment of relapsed or refractory peripheral T-cell lymphoma based on ORR shown in phase II, single-arm studies. Pralatrexate is an antifolate investigated in 115 patients, 109 of whom were evaluable(37). ORR was 29% (95% CI, 21-39%), with 12 CRs (11%) and 20 PRs (18%). Median duration of response was 10.1 months (range 1-673 days), median PFS was 3.5 months, and median OS 14.5 months. Seventy-four percent of patients had a grade 3 or 4 adverse event, most of which hematological (19% grade 4 thrombocytopenia, 16% grade 3 anemia, 14% grade 3 thrombocytopenia and neutropenia).

Belinostat is a histone deacetylase inhibitor (HDACi) studied in 129 patients, 120 of whom were evaluable(38). ORR was 26%, with 12 CRs (10%) and 19 PRs (16%). Median duration of response was 8.4 months (range NR-36 months). Median PFS was 1.6 months, and median OS was 7.9 months. Sixty-one percent of patients had a grade 3 or 4 adverse event, 22 patients died during treatment, 10 of whom (7.8%) due to treatment-emergent adverse events within 30 days of the last belinostat dose.

A recent meta-analysis of available treatments for relapsed/refractory PTCL showed that, even though approved, belinostat and pralatrexate were less effective and safe than several other regimens, including single-agent alemtuzumab (**Figure 4**). Most effective was the anti-CD30 immunotoxin brentuximab vedotin in anaplastic large cell lymphoma (ORR 86%), though when given to unselected patients with PTCL-NOS the ORR of 41% was similar to that of alemtuzumab (36%)(39).

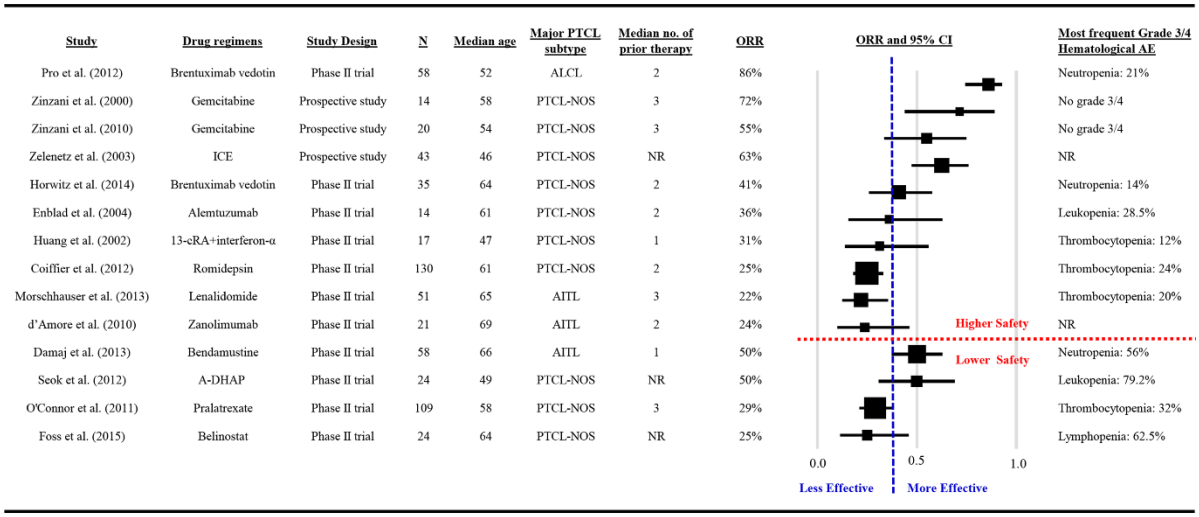

Figure 4: Safety and efficacy of agents used in treatment of relapsed/refractory PTCL(**39**)

1.2.3.2 Alemtuzumab in relapsed/refractory PTCL

In a phase II European study of 14 patients with relapsed/refractory disease (2 AITL, 2 angiocentric, 10 PTCL-NOS) ORR was 36%, with 3 CRs (all with PTCL-NOS), and 2 PRs (1 angiocentric, 1 PTCL-NOS)(40). Duration of the complete responses was 2, 6, and 12 months. Six patients had CMV reactivation, two had pulmonary aspergillosis, and four had pancytopenia (including two patients with HLH). Overall, five of the 14 patients died of infectious complications. Based on this study, alemtuzumab is one of the NCCN-recommended alternative salvage regimens for patients with PTCL (both angioimmunoblastic and NOS) who are not candidates for an allogeneic stem cell transplant(41).

1.2.3.3 Treatment of relapsed/refractory CTCL

There are four FDA-approved drugs for relapsed/refractory CTCL currently on the market: bexaroten, romidepsin, vorinostat, and brentuximab vedotin. The fifth, anti-CD25 immunotoxin denilekin difitox, was withdrawn by the manufacturer and is no longer available. A phase III placebo-controlled trial of the drug in 144 patients with $CD25^+$ CTCL showed ORR of 44% (10% CRs, 34% PRs), compared to 15.9% in the placebo-treated group(42). Two thirds of the patients had early-stage disease (≤IIA), and only 6.3% had Sézary syndrome (SS). Patients with visceral involvement and lymph node status ≥LN3 were excluded.

The RXR-selective retinoid bexaroten was given at two different dose levels to 94 patients with stage ≥IIB CTCL, of whom 31% had erythroderma and 10% had visceral involvement(43). Only 16% of patients had ≥15% circulating Sézary cells, though 27% had the cells detectable. ORR was 45-55%, with 1-5 CRs. One of five patients with circulating Sézary cells had a substantial decrease in counts. Six of 19 patients (32%) with generalized erythroderma, and four of 17 (24%) with SS had a response. The most common grade 3 or 4 AEs were hyperlipidemia (34-45%) and pruritus (8-14%). One of 17 deaths was judged to be possibly drug related, that of a patient who developed liver failure with coagulopathy.

The HDAC inhibitor vorinostat was investigated in a phase IIB multicenter trial of 74 patients, 61 with advanced (stage ≥IIB) disease, including 30 with SS(44). ORR was 29.7% (1 CR, 21 PRs), including 10/30 patients with SS. Grade 3 or 4 AEs were seen in 28% of the patients, with

the most common being fatigue (5%), pulmonary embolism (5%), thrombocytopenia (5%), and nausea (4%).

Another HDAC inhibitor, romidepsin, was investigated in two concurrent phase II trials which included a total of 167 patients, 130 of whom with stage ≥IIB disease(45, 46). ORR was 34-38%, and a total of 10 patients (6%) had CRs, three of whom had SS. Reports of grade 3 or 4 AEs varied between the studies, with lymphopenia ranging from 21% to <10%, neutropenia 14% to <10%, and thrombocytopenia 6% to none. There were 10 deaths within 30 days of receiving the last dose of the drug, two from sepsis, one from hypertrophic cardiac disease, and the rest from disease progression.

Chemotherapy, including CHOP-based regimens, has high response rates (50-88%), but duration of response <6 months and high toxicity(47). More recently, results of a phase III trial of brenutixmab vedotin compared to methotrexate or bexaroten in $CD30^+$ CTCL were reported as positive for improvement in global overall response lasting at least 4 months (ORR4), which was 56.3% v 12.5% after a median follow-up of 22.9 months(48).

1.2.3.4 Alemtuzumab in relapsed/refractory CTCL

The first reported prospective trial of alemtuzumab in CTCL was performed in Europe with 30 mg given intravenously for 12 weeks to 22 patients, 7 of whom with circulating Sézary cells(31). CD52 expression was confirmed by flow cytometry. ORR was 55% (7 CRs, 5 PRs), with median duration of response 12 months (range 5-32+).

Subsequent trials showed alemtuzumab was more effective in patients with circulating Sézary cells (B1-2) or erythrodermia (T4)(47). In a prospective trial, alemtuzumab 30 mg was given intravenously three times weekly for 4 weeks, then subcutaneously for 8 weeks to 19 patients with stage III-IV, T4 disease (one of whom had visceral metastases)(49). ORR was 84% (9 CRs, 7 PRs), median OS 41 months, PFS 6 months. There was minimal residual disease present in 11/16 patients with response.

In another prospective study, alemtuzumab 10 mg was given subcutaneously three times weekly for 6+ weeks to 18 patients with “leukemic” CTCL and two with MF (without circulating cells)(50). For L-CTCL ORR was 100% (16 CRs, 2 PRs) in blood, and 89% (8 CRs, 8 PRs) in skin. There were no responses in either of the two patients with MF.

1.2.3.5 Treatment of relapsed/refractory T-cell prolymphocytic leukemia (T-PLL)

Similar to ATL, T-PLL is an aggressive mature T-cell malignancy which lacks approved first or second-line treatments(51). In a phase I trial of the purine nucleoside analogue nelarabine, the ORR was 20% for nelarabine alone, and 63% (13% CR) when combined with fludarabine(52). Retrospective data from 9 European centers showed ORR 76% with 60% CR and 16% PR when Alemtuzumab was given to 38 patients intravenously until maximal response or intolerance for median 5 weeks (range 2-82 days)(26). Median disease-free interval was 7 months (4-45 months). Of 12 patients retreated with Alemtuzumab following relapse, 42% achieved a second CR, and one a PR, with responses lasting 5-6 months. Median overall survival was 10 months overall, 16 months in patients who achieved CR, 9 months in those achieving PR, and 4 months in non-responders.

1.2.4 Interleukin-15

IL-15 is a 14-15 kDa member of the 4-alpha helix bundle family of cytokines that acts through a heterotrimeric receptor involving IL-2/IL-15R beta subunit shared with IL-2, the common

gamma chain (γc) shared with IL-2, IL-4, IL-9, IL-21 and the IL-15 specific receptor subunit IL-15R alpha (CD215)(53-60). Whereas IL-15 and IL-2 also have a number of similarities as activators of NK cells, CD4+ and CD8+ effector T cells, there are a number of biological features distinctive to each of the cytokines(57, 61, 62). There are a number of functional differences for the 2 cytokines which is first evident in their distinctive secretory process. Secreted IL-2 engages preformed heterotrimeric receptors expressed in cis on activated T and B cells. In contrast, IL-15 acts as a cell-surface molecule as part of an immunological synapse with IL-15 and IL-15Rα produced in trans on adjacent mononuclear cells (MNC) like monocytes and DCs which have been stimulated by interferon, and/or CD40 ligation or Toll-like receptor activation. IL-15 binds to IL-15Rα with high-affinity Kd > 10-11 M and the co-expressed IL-15 and IL-15Rα remain together with the capacity to recycle through endosomal vesicles and re-emerge appear on the MNC cell surfaces so that the combination is co-expressed for many days. Dubois and coworkers have demonstrated that this heterodimer (IL-15 and IL-15Rα) stimulate primarily effector NK and CD8 T-cells that express IL-2/IL-15Receptor beta (β) chain (CD122) and the γc (CD132) but do not express IL-15Rα(63).

1.2.4.1 Interleukin 15 as an Immunotherapeutic Agent

In the now more than two decades of clinical investigation into immunotherapeutics for the treatment of human malignancies IL-2 remains the prototypic agent. IL-2 has been approved by the FDA for the treatment of malignant melanoma and renal cell cancer(61). While IL-2 has advantageous features shown to contribute to the *in vivo* antitumor immune response, there are less than desirable consequences of high-dose IL-2 (HD IL-2) treatment such as the capillary leak syndrome, hypotension, the previously cited role in AICD and the maintenance of Tregs. Like IL-2, IL-15 has been shown in many model systems to be a portent stimulator of T and NK-cell functions but IL-15 lacks a number of IL-2's negative characteristics that suggest that IL-15 may have greater clinical potential as an immunotherapeutic in oncology(59, 60).

1.2.4.2 Interleukin-15 in Preclinical Immunotherapy Models

A number of studies in murine models suggested that IL-15 may prove to be of value in the therapy of neoplasia(64-70). In particular, whereas following intravenous administration of MC38 syngeneic colon carcinoma cells wild-type mice died of pulmonary metastases within 6 weeks, IL-15 transgenic mice survived for more than 8 months following infusion of the MC38 tumor cells. Furthermore, Klebanoff and coworkers demonstrated that IL-15 enhanced the *in vivo* activity of tumor related CD8+ T-cells in the T-cell receptor transgenic mouse (pmel-1) whose $CD8^+$ T-cells recognized an epitope derived from the melanoma antigen gp100(65). In our studies with Yu and Zhang IL-15 was shown to prolong the survival of mice with established DC26 and MC38 colon cancers and with TRAMP-C2 prostatic cancer(65, 68, 70).

On the basis of the animal and laboratory trials of IL-15, great interest was generated among leading immunotherapeutic experts participating in the NCI Immunotherapy Agent Workshop who ranked IL-15 as the most promising unavailable immunotherapeutic agent to be brought to therapeutic trials(71).

1.2.4.3 Toxicity, Pharmacokinetics, Immunogenicity and Impact on Elements of the Normal Immune System of Recombinant IL-15 in Rhesus Macaques

The safety of IL-15 was evaluated in mice and rhesus macaques(72-74). Müller et al. injected SIV-infected rhesus macaques with recombinant IL-15 at doses of 10 mg or 100 mg/kg twice weekly subcutaneously for 4 weeks(73). IL-15 induced a nearly 3-fold increase in the number of peripheral $CD8^+$ $CD3^{-/-}$ NK cells. All clinical laboratory results remained within normal limits

with the exception of a non-significant increase in the number of circulating platelets. Berger et al. administered human recombinant IL-15 to rhesus macaques at doses of 2.5 -15 mcg/kg daily by subcutaneous injection for up to 14 days(72). Daily administration of IL-15 for up to 14 days caused reversible severe neutropenia, anemia, weight loss, generalized skin rash and a massive expansion of T cells. The bone marrow of a single neutropenic animal was found to be hypocellular. Recombinant human IL-15 (Escherichia coli produced rhIL-15), produced by the Biopharmaceutical Development Program, NCI, was administered at a dosing schedule of 12 daily intravenous bolus infusions at doses of 10, 20 and 50 mcg/kg/day to rhesus macaques(74). The only biological meaningful laboratory abnormality was a grade 3/4 transient neutropenia. This neutropenia was shown to be secondary to a redistribution of neutrophils in that bone marrow examinations demonstrated increased marrow cellularity including cells of the neutrophil series. Furthermore, neutrophils were observed in sinusoids of markedly enlarged livers and of the spleen suggesting IL-15 mediated neutrophil margination or redistribution from the circulation to the tissues. Verri and coworkers demonstrated that following intraperitoneal administration of IL-15 there was a cascade of cytokines in the order of IL-18, MIP-1 alpha, MIP-1 beta and TNF alpha that was associated with a redistribution of neutrophils from the circulation to the peritoneal cavity(75).

A 12-day bolus of intravenous administration of 20 mcg/kg/day of IL-15 to rhesus macaques was associated with a 4-to-8-fold increase in the number of circulating NK, stem, central and effector memory CD8 T-cells(74). Subsequently alternative routes of administration were evaluated in rhesus macaques including continuous intravenous infusion (CIV) and subcutaneous administration of IL-15. The administration of IL-15 by CIV at 20 mcg/kg/day for 10 days led to an approximately 10-fold increase in the number of circulating NK cells and a massive 80-to100-fold increase in the number of circulating effector memory CD8 T-cells(76) (**Figure 5**).

Subcutaneous infusions at 20 mcg/kg/day for 10 days led to a more modest 10-fold expansion in the number of circulating effector memory CD8 T-cells. No vascular leak syndrome, hemodynamic instability or renal failure was observed in these studies. In addition, no animal developed autoimmune disorders. Two repeat courses of subcutaneous IL-15 were largely without adverse effects.

IL-15 Administration to Rhesus Macaques

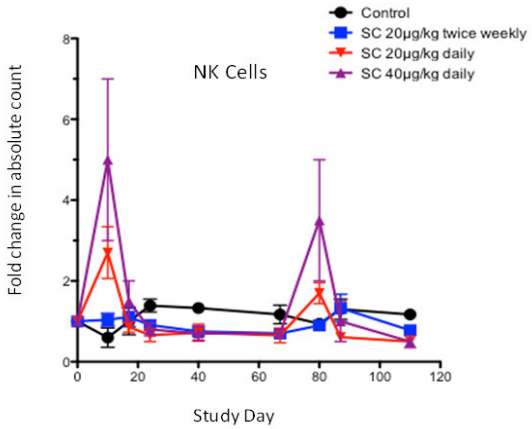

Figure 5: Impact of s.c. IL-15 administration to rhesus macaques on fold change in the absolute number of circulating NK cell

The Biopharmaceutical Development Program (BDP) of the NCI has produced a single chain recombinant human IL-15 (sch rhIL-15) synthesized in an Escherichia coli (E. coli) system and purified into clinical grade reagent satisfactory for human trials(77). Toxicology studies in non-human primate (NHP) rhesus macaques have identified schedules for intravenous and subcutaneous routes of administration that have been or are being evaluated in phase I clinical trials. The First in Human (FIH) trial that administered rhIL-15 as 30 minute infusion (intravenous bolus [IVB]) daily for 12 consecutive days has been completed. Two additional trials are currently assessing continuous IV (CIV) treatment for 10 days (240 hours) every 6 weeks or s.c. administration Monday through Friday for 2 consecutive weeks are nearing completion after defining the maximum tolerated dose (MTD) for these parallel treatments(77, 78).

1.2.4.4 Most advantageous rhIL-15 treatment schedule to be combined with alemtuzumab
In the initial rhIL-15 IVB trial acute toxicities that began 2 to 2 ½ hours after the 30 minute infusion including decreased blood pressure requiring significant amounts of IV fluid support, temperature spikes not completely responsive to the addition of scheduled antipyretics and rigors likely related to intense cytokine production limited dose escalation and immune activation. These events seemed related to the high IL-15 C_{max} levels and were felt to be due to stimulation of cells that expressed only the IL-2/IL-15R beta and γc receptor. At roughly the same time these toxicities were being better understood through analyzing the data from the trial and data from the macaques' toxicology studies evaluating s.c. and CIV schedules. Data from the NHP showed a more favorable pharmacokinetic profile for the s.c. and CIV schedules that did not have extremely elevated early IL-15 levels and produced better lymphocytes expansions than the IVB schedule(76).

In the first in-human trial using Escherichia coli produced recombinant IL-15 (rhIL-15) evaluating patients with metastatic malignant melanoma or metastatic renal cell cancer, subjects were administered IV bolus injections of rhIL-15 at doses of 3.0, 1.0 and 0.3 mcg/kg per day for 12 consecutive days(77). A total of 18 patients were treated; 5 patients received 3.0 mcg/kg per day, 4 patients received 1.0 mcg/kg per day, and 9 patients received 0.3 mcg/kg per day for 12 consecutive days. Of these patients, 11 had a primary diagnosis of metastatic melanoma and 7 had a primary diagnosis of metastatic renal cell carcinoma.

The maximum tolerated dose was determined to be 0.3 mcg/kg per day. Dose limiting toxicities noted at the 1.0 mcg/kg per day and 3.0 mcg/kg per day doses included Grade 3 hypotension, thrombocytopenia and elevations of ALT and AST.

Flow cytometry of peripheral blood lymphocytes revealed an efflux of NK and memory CD8 T-cells from the circulating blood within 30 minutes upon IL-15 administration, followed by influx and hyperproliferation yielding a 10-fold expansion of NK cells that ultimately returned to baseline (**Figure 6**). Furthermore, there were significant increases in the gamma delta and CD8 memory T-cells.

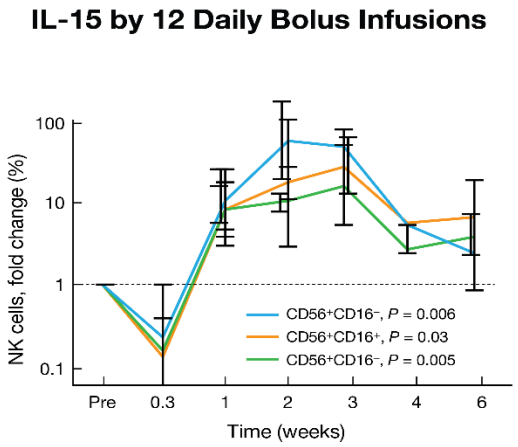

Figure 6: Effect of 12-daily bolus infusions of IL-15 on the fold change in the number of circulating diverse subsets of NK cells in patients with metastatic malignancy.

In this first in-human Phase I study there was no responses with stable disease as the best response. However, 5 patients manifested a decrease between 10 and 30 percent in their marker lesions and 2 patients had clearing of lung lesions.

1.2.4.5 A Phase I Study of Subcutaneous Recombinant Human IL-15 (rhIL-15) in Adults with Metastatic Cancer (NCT01727076)

Looking for an alternative dosing strategy, we went on to complete a Phase I trial of subcutaneous recombinant human IL-15 in cycles consisting of 5 daily injections of rhIL-15 given Monday-Friday for 2 weeks, then 2 weeks of observation with potential for additional cycles. Three patients each were enrolled in 0.25, 0.5, 1.0, and 2.0 mcg/kg/day dose levels and six patients were evaluated at 3.0 mcg/kg/day. Eighteen patients completed at least one cycle with one DLT at 3.0 mcg/kg/day and one serious adverse event, pancreatitis, at 2.0 mcg/kg/day (see **Figure 7** for other AEs with s.c. IL-15). Flow-cytometry data indicated a consistent increase in the frequency of CD56bright CD3- negative NK cells peaking at day 15 (day 12 = last dose). The mean fold increase with 3 mcg/kg/day of IL-15 in circulating NK cell numbers peaked at 10.8-fold. The maximum fold increase in circulating CD8 + T cells was 3.3-fold. It was concluded that subcutaneous IL-15 was well tolerated and that **the 2 mcg/kg/day dose level was the recommended phase II dose to be pursued in combination trials**.(79)

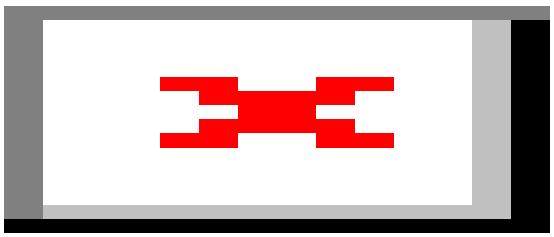

Figure 7: CITN11-02 Adverse Events

Laboratory abnormalities in both this and the CIV trial(80) have been very similar to IVB rhIL-15 trial with no significant biochemical changes other than mild hypoalbuminemia and or elevated liver function tests. Hematologic abnormalities have included early transient lymphopenia and later moderate expansion of lymphocytes. Preliminary results indicate that the increases in the ALC are primarily driven by expansion in NK cell rather than T-cell numbers. The best clinical response to date in any subjects from either protocol has been stable disease, but that includes disease stability persisting in a few subjects after discontinuation of rhIL-15 treatment. The combined safety experience from the s.c. and CIV rhIL-15 trials has generally demonstrated mild clinical and laboratory side effects typically seen with cytokine treatment that resolved quickly after discontinuation of treatment (78, 79, 81). The SAEs seem to be isolated events and do not reveal a pattern of toxicity or a potential for autoimmune toxicities that would preclude the plan for evaluation of higher doses of rhIL-15.

1.2.4.6 The lack of effect of IL-15 on acute ATL patients' leukemic cells

We have performed a number of studies that support our view that IL-15 will not induce proliferation of acute ATL patients' cells and therefore represents minimal risk: (1) Over 60 patients have been treated with IL-15 with no evidence that it stimulates the proliferation of malignant cells; (2) When 5 nanograms/mL (the highest concentration achieved with subcutaneous or continuous intravenous infusions of IL-15 in human clinical trials) and 50 nanograms/mL (10-fold greater than the IL-15 achieved in s.c. human trials) to *ex vivo* PBMCs from patients with acute ATL there was no evidence of proliferation; (3) When the same concentrations of IL-15 were added to five cytokine independent ATL cell lines that reflect the state of acute ATL in 48-hour cultures with proliferation assessed by tritiated thymidine the addition of 5 and 50 nanograms per mL decreased proliferation in one cell line, had minimal stimulation in two and no effect on the remaining two ATL cell lines; (4) In an additional study following injection of the cytokine independent ATL cell line MET-1 into the SCID/non-obese diabetic (NOD) mice the administration of IL-15 to these ATL-bearing mice inhibited rather than stimulated the proliferation of these cells, thereby resulting in an increase in the survival of the ATL cell-bearing mice (**Figure 8**). Finally, the IL-15 administration in the proposed trial will be followed by alemtuzumab administration that we have shown kills ATL cells. Taken as a whole, IL-15 did not augment the proliferation of acute ATL cells and thus does not represent an increased risk.

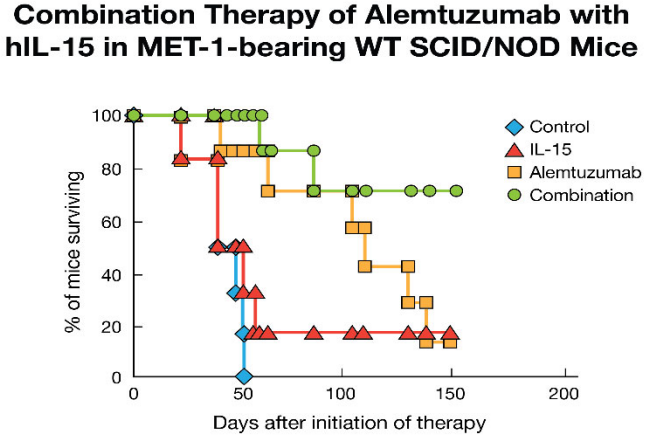

Figure 8: The impact of combination therapy of alemtuzumab with IL-15 in the MET-I (ATL) bearing SCID/NOD mouse.

STATISTICS:

A comparison of the survival curves by log-rank (Mantel-Cox) tests the p value = 0.07. Comparing the survival rates between the two groups at the end of the study using Fisher's exact test the p value of the two-sided test is 0.10. Although the difference is not statistically significant (P > 0.05), the 95% confidence interval shows a trend of survival benefit for alemtuzumab + IL-15. The study is being expanded with a larger number of animals.

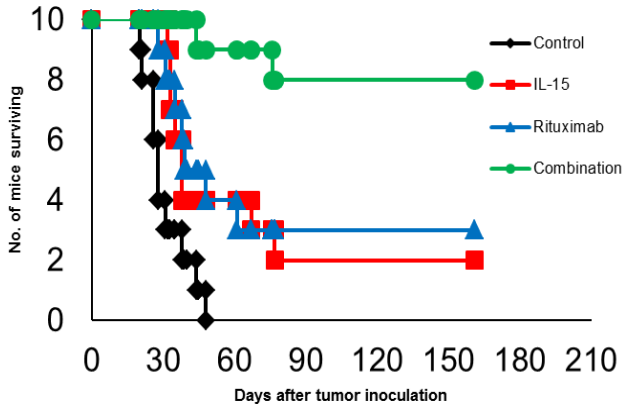

Figure 9: Rituximab combined with hIL-15 in a syngeneic mouse model inoculated i.v. with EL4 cells transfected with human CD20

1.2.4.7 Preclinical Trials of IL-15 with Anticancer Monoclonal Antibodies to Augment their ADCC

While the *in vivo* effects of rhIL-15 in cancer patients are still not entirely clear, the initial clinical data has demonstrated some important and positive findings. Nevertheless, it is clear that to achieve its potential in the treatment of cancer IL-15 will have to be used in combination with other therapeutic agents(78, 79, 82-85). Treatment with rhIL-15 has caused dramatic expansion of circulating NK cells in the patients treated with the IVB, s.c. and CIV regimens(77, 78) (Waldmann TA and Conlon KC, unpublished observations). Coupled with the data from the preclinical animal model experiments demonstrating the capacity of rhIL-15 to increase the number of activated NK cells, T-cells and monocytes, this information supports administering rhIL-15 with antitumor monoclonal antibodies (MoAbs) to augment their ADCC against tumor cells. To further investigate this strategy, our laboratory has used an immunocompetent syngeneic mouse model of B-cell lymphoma to investigate the combination of IL-15 and rituximab (Wen B, Zhang M and Waldmann TA, unpublished observations). Wild-type CD57BL/6 mice (N = 40) were inoculated intravenously with EL4-CD20 cells, a mouse lymphoma line transfected with human CD20 and the mice were distributed into 4 treatment groups (control, IL-15 alone, rituximab alone and the combination) of 10 mice each. IL-15 (5 µg/mouse) was administered 5 x per week for 4 weeks beginning 3 days after EL4-CD20 inoculation. In the cohorts receiving rituximab, the MoAb (100 µg/mouse) was given once per week for 4 weeks starting on 5 days after EL4-CD20 inoculation. The mice were monitored for tumor-related death after completion of the treatment period. IL-15 or rituximab monotherapy

prolonged survival of mice when compared to the control group ($P < 0.05$), but the combination of IL-15 and rituximab showed the greatest prolongation of survival compared to monotherapies (< 0.01). As seen in **Figure 9** so that 75 days after tumor inoculation 90% of the combination treatment group were still alive, in contrast 30% survival for monotherapy groups and no surviving mice in the control group.

We also identified a role for IL-15 in augmenting ADCC by performing an *ex vivo* experiment using NK cells purified from spleens from either wild-type (WT) or FcR$\gamma^{-/-}$ mice treated with IL-15 five days before extraction. NK cells were cultured in triplicate with ^{5}Cr labeled EL4-CD20 cells with or without rituximab and lysis was evaluated using a chromium-51 release assay. NK cells from IL-15 treated WT mice combined with rituximab resulted in a 3-fold increase in ADCC when compared to the monotherapies alone (16% combination versus 6% IL-15 and 5% rituximab at a 10:1 E:T ratio). In contrast, NK cells from FcR$\gamma^{-/-}$ showed no significant increase in ADCCs when treated with rituximab (0.9% rituximab versus 1.1% control and 3.6% combination versus 3.6% IL-15 at a 10: E:T ratio). In agreement with previous research this study provides further evidence that IL-15 increases the efficacy of rituximab primarily through enhancing monocyte and NK-cell mediated ADCC.

In a parallel preclinical trial, we administered a combination therapy of alemtuzumab with rhIL-15 in our MET-1 bearing xenograft model in wild-type SCID/NOD mice (**Figure 5**).

In this study MET-1 human HTLV-1 adult T-cells were administered intravenously to WT SCID/NOD mice. Therapy was initiated when the surrogate marker human serum sIL-2R alpha reached 1,000-10,000 units/mL that is when the tumor was fully established. All mice in the control group died of tumor by day 50. In mice receiving IL-15 the median 50% survival was by day 50, with 20% of the mice persisting for the 150 days of the study. In mice receiving alemtuzumab there was a 50% survival of the mice at day 105, with 20% of the mice surviving to day 150. In contrast the 50% mice survival point was not achieved at 21weeks in animals receiving the combination of IL-15 and alemtuzumab, with 70% of the mice surviving at 150 days after initiation of therapy.

In summary of prior studies, the administration of alemtuzumab to patients with adult T- cell leukemia was associated with a PR or CR in 52% of the patients, however the responses were short-lived. The clinical trials in humans indicate that subcutaneously administered IL-15 is well tolerated and is associated with an increase in the number of natural killer cells. Finally, studies combining the monoclonal antibody, alemtuzumab with rhIL-15 in a xenograft murine tumor model provided the scientific support for the combination of alemtuzumab with rhIL-15 in patients with chronic and acute leukemic forms of ATL. The dose and dosing scheme proposed for alemtuzumab is the widely accepted dose and dosing strategy and is the one that was used previously at the NCI in our clinical trial of patients with ATL. The dosing strategies with rhIL-15 are identical to those used in the clinical trial performed in conjunction with the Cancer Immunotherapy Network that evaluated s.c. IL-15 daily for days 1 through 5 and 8-12(77, 78).

1.2.5 Addition of Venous Thromboembolism (VTE) Prophylaxis

As of July 2019, eight patients have been treated on this study, two of whom have developed grade 3 VTE. In both cases VTE was manifested as pulmonary embolism (PE) of the lobar pulmonary artery, which was asymptomatic and found incidentally on a restaging CT scan performed on Week 6 Day 5, the last day of alemtuzumab treatment. In one case there was also a finding of a central venous catheter-associated thrombus. The expected incidence of grade ≥3

VTE with alemtuzumab treatment is 4.4% (86), and the two occurrences of PE raise concerns for IL-15 increasing the risk of VTE. However, in both cases the Week 3 Day 5 CT, performed after all IL-15 doses and the first three doses of alemtuzumab were administered, showed no evidence of PE, making IL-15 contribution unlikely. It is possible that the type and position of the central venous catheter through which alemtuzumab was administered contributed to the increased risk, as patients receiving alemtuzumab via a port did not experience VTE. Because of the suspected increased incidence of VTE, appropriate prophylaxis will be initiated per Section **4.4** and additional protocol stopping rules applied per Section **3.1.2.1**.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Age ≥ 18 years, no upper age limit.

2.1.1.2 Patients diagnosed with a leukemia or lymphoma as follows:

- Chronic or acute leukemia forms of HTLV-1 associated adult T-cell leukemia (see **Appendix B**);
- Peripheral T-cell lymphoma (angioimmunoblastic, hepatosplenic, or not otherwise specified); or,
- Cutaneous T-cell lymphoma stage III or IV with circulating monoclonal cells (B1 or B2) and/or erythrodermia (T4)
- T-cell prolymphocytic leukemia (T-PLL)

NOTE: Diagnosis must be validated by the Pathology Department, NCI.

2.1.1.3 Patients must have measurable or evaluable disease.

NOTE: All patients with greater than 10% abnormal CD4+ homogeneous $CD3^{low}$ strongly CD25+ expressing cells, or greater than 5% Sézary/T-PLL cells, among the PBMCs in the peripheral blood will be deemed to have evaluable disease.

2.1.1.4 Abnormal T cells must be $CD52^{+}$ as assessed by flow cytometry or immunohistochemistry.

2.1.1.5 Patients must have a life expectancy of ≥ 2 months

2.1.1.6 Patients must have been refractory or relapsed following front-line therapy; those with CTCL or PTCL who have $CD30^{+}$ disease must have progressed during or after treatment with brentuximab vedotin or are unable to receive treatment due to allergy or intolerance.

2.1.1.7 Patients must have recovered to less than grade 1 or to baseline from toxicity of prior chemotherapy or biologic therapy and must not have had major surgery, chemotherapy, radiation or biologic therapy within 2 weeks prior to beginning treatment. **NOTE:** Exceptions to this include events not considered to place the subject at unacceptable risk of participation in the opinion of the PI (e.g., alopecia).

2.1.1.8 DLCO/VA and FEV – 1.0 > 50% of predicted on pulmonary function tests.

2.1.1.9 Adequate laboratory parameters, as follows:

- Serum creatinine of ≤ 1.5 x the upper limit of normal
- AST and ALT < 3 x the upper limit of normal
- Absolute neutrophil count ≥ 1,500/mm^3 and platelets ≥ 100,000/mm^3

2.1.1.10 ECOG ≤ 1 (see **Appendix A**).

2.1.1.11 Patients must be able to understand and sign an Informed Consent Form.

2.1.1.12 All patients must use adequate contraception during participation in this trial and for 4 months following completing therapy.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who have received any systemic corticosteroid therapy within 4 weeks prior to the start of therapy, or 12 weeks if given to treat graft versus host disease (GVHD), with the exception of physiological replacement doses of cortisone acetate or equivalent.

2.1.2.2 Patients who have undergone allogeneic stem cell transplantation and have required systemic treatment for GVHD (including but not limited to oral or parenteral corticosteroids, ibrutinib, and extracorporeal phototherapy) within the last 12 weeks

2.1.2.3 Clinical evidence of (parenchymal or meningeal) CNS involvement or metastasis. In subjects suspected of having CNS disease, a magnetic resonance imaging (MRI) scan of the brain and lumbar puncture should be done to confirm.

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

- Positive hepatitis B serology indicative of previous immunization (i.e., HBsAb positive and HBcAb negative) or a fully resolved acute hepatitis B infection is not an exclusion criterion.
- If hepatitis C antibody test is positive, then the patient must be tested for the presence of HCV by RT-PCR and be HCV RNA negative.

NOTE: HIV-positive patients are excluded from the study. Alemtuzumab may produce a different pattern of toxicities in patients with HIV infection; in addition, the depletion of T cells produced by alemtuzumab may have adverse effects on HIV-positive individuals.

2.1.2.5 Concurrent anticancer therapy (including other investigational agents).

2.1.2.6 History of severe asthma or presently on chronic inhaled corticosteroid medications (patients with a history of mild asthma not requiring corticosteroid therapy are eligible).

2.1.2.7 Patients with smoldering and lymphomatous ATL.

2.1.2.8 Pregnant or nursing patients.

2.1.2.9 Patients who have previously received alemtuzumab are ineligible. **NOTE:** Patients with relapsed T-PLL who have achieved at least a partial response to prior alemtuzumab are eligible.

2.1.2.10 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, moderate/severe graft versus host disease, cognitive impairment, active substance abuse, or psychiatric illness/social situations that, in the view of the Investigator, would preclude safe treatment or the ability to give informed consent and limit compliance with study requirements.

2.1.3 Recruitment Strategies

Patient accrual will be facilitated by a contract with the University of the West Indies (Jamaica) that refers patients with ATL and from the population of patients screened in the lymphoid malignancy clinics of the National Institutes of Health. These will include both referrals from outside physicians as well as patient self-referrals. In addition, we participate in a locoregional consortium of eight academic institutions within the mid-Atlantic region that shares information regarding active clinical protocols and aims to enhance patient recruitment across the region. In addition, this study will be posted on NIH websites and NIH Social Media forums.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed after a consent for screening has been signed

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols). Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Screening evaluation must be performed within 28 days prior to study enrollment, except as indicated below.

- Diagnosis of leukemia/lymphoma confirmed by the Laboratory of Pathology, NCI. Pathological confirmation of diagnosis can be carried out on available slides from either primary screen or biopsy performed either at the Clinical Center or outside institutions (no time restriction).
- Flow cytometry to assess CD52 and 1) $CD3^{low}CD4^{+}$ $CD25^{hi}$ (for patients with ATL), or 2) $CD4^{+}$ $CCR4^{+}$ $CD27^{+}$ (for patients with CTCL) in the peripheral blood cells and lymphocyte sets and subsets. Send one 10 mL lavender top tube to the Immunology Service of the Department of Laboratory Medicine. See Section **5.1** for additional collection times.
- Complete medical history and physical exam with documentation of sites of disease.

- Documentation of ECOG Performance Score (see **Appendix A**).
- Vital signs (VS): heart rate, blood pressure, respiration, temperature and oxygen saturation (SO2) by transcutaneous pulse oximeter.
- PET scan of the torso and CT scan of the chest, abdomen, pelvis and neck or MRI if CT scan is not sufficient or contraindicated. Other imaging studies may be done to document disease as appropriate and at the direction of the PI.
- Complete blood count (CBC) with differential.
- Acute Care Panel (sodium, potassium, chloride, total CO2, glucose, creatinine, BUN) Hepatic Panel (alkaline phosphatase, ALT, AST, total and direct bilirubin), LDH and Mineral Panel (serum calcium, phosphorous, magnesium, albumin)
- Serum pregnancy test in women of childbearing potential.
- Hepatitis B Surface Antigen, Hepatitis B Surface Antibody, Hepatitis B Core Antibody, and Hepatitis C. HIV 1 /2, and HTLV-1/2 serologies.
- EKG
- Pulmonary function tests with DLCO.
- Echocardiogram with measurement of left ventricular ejection fraction (LVEF).
- MRI scan of the brain and lumbar puncture for patients with neurological symptoms or signs.
- Skin biopsy of clinically suspicious lesions. One to three lesions may be biopsied if necessary to confirm the diagnosis of ATL or CTCL.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found **here**.

2.3.1 Treatment Assignment Procedures

2.3.1.1 Cohorts

Number	Name	Description
1	Leukemia or lymphoma	Patients with a T-cell leukemia or lymphoma (i.e., chronic or acute HTLV-1 associated ATL; peripheral T-cell lymphoma; cutaneous T-cell lymphoma; or, T-cell prolymphocytic leukemia [up to 30 patients])

2.3.1.2 Arms

Number	Name	Description
1A	Experimental: Dose Escalation	IL-15 for 10 doses over two weeks followed by alemtuzumab for 4 weeks per dosing schema to determine the maximum tolerated dose (MTD)
1B	Experimental: Dose Expansion	IL-15 for 10 doses over two weeks followed by alemtuzumab for 4 weeks at the maximum tolerated dose (MTD)

2.3.1.3 Treatment Assignment

Treatment assignment is single arm, open-label, and non-randomized/non-stratified (i.e., subjects in Cohort 1 directly assigned to Arm 1 based on the dose level open to enrollment).

2.4 BASELINE EVALUATION

The following tests/procedures should be performed within 28 days prior to initiating treatment unless otherwise noted. It is not necessary to repeat test that were done for screening if they were done within 28 days prior to initiating treatment.

2.4.1 Clinical Evaluations

- Complete medical history and physical exam with documentation of sites of disease.
- Documentation of ECOG Performance Score (see **Appendix A**).
- Vital signs: heart rate, blood pressure, respiration, temperature and oxygen saturation (SO2) by transcutaneous pulse oximeter.
- Height and Weight

2.4.2 Laboratory Evaluations

- Serum pregnancy test in women of childbearing potential, repeated within 3 days prior to starting the study drug.
- Thyroid function tests
- Troponin
- Urinalysis
- CBC with differential and reticulocyte count
- Acute Care Panel (sodium, potassium, chloride, total CO2, glucose, creatinine, BUN) Hepatic Panel (alkaline phosphatase, ALT, AST, total and direct bilirubin), LDH and Mineral Panel (serum calcium, phosphorous, magnesium, albumin)
- Serum Lipase and Amylase
- Glucose-6 phosphate dehydrogenase (G-6PD)
- Antinuclear antibody (ANA), rheumatoid factor (RF) and anti-thyroid antibody
- CMV/EBV PCR; Quantitative, Blood
- HLA typing (A, B, C, DR, DQ)

2.4.3 Imaging Studies

Every participant should have an evaluation of known sites of disease as part of baseline evaluation. NOTE: Only results from NIH are accepted.

- One or more of the following studies: CT, MRI, FDG-PET and/or clinical photography
- Patients with neurological symptoms or signs should undergo MRI scan* of the brain and lumbar puncture

*NOTE: The MRIs to be done in this study may involve the use of the contrast agent gadolinium, if clinically indicated.

2.4.4 Other Procedures

- Bone marrow biopsy and aspiration to assess for disease involvement (not needed if one has already been performed at the NIH Clinical Center within 1 year prior to enrollment).
- All patients with cutaneous disease will have their disease assessed by a dermatologist using the modified Severity Weighted Assessment Tool [Appendix I].
- Selected patients with cutaneous disease will have clinical photography performed to document their skin disease.

2.4.5 Research Correlates

See Section **5** for additional information.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a single institution nonrandomized, IL-15 dose-escalation Phase I study evaluating the combination of s.c. rhIL-15 with alemtuzumab in patients with relapsed or refractory ATL, PTCL, T-PLL, or CTCL where both escalating doses of rhIL-15 and sequential administration of alemtuzumab will be administered.

rhIL-15 will be given as a subcutaneous (s.c.) injection once daily Monday-Friday for a total of 10 doses.

On Day 1 (or before if clinically indicated) of Week 3, patients will be admitted to the NIH Clinical Center inpatient unit for the alemtuzumab infusions. Patients may be discharged from the inpatient unit and treated in the outpatient clinic when their experience with the treatment indicates that the alemtuzumab can be given safely in an outpatient setting. **During week 3, all patients will receive standard initial (tachyphylaxis) dosing of alemtuzumab by 2-hour intravenous infusion: Day 1→ 3 mg, Day 2 →10 mg, and Day 3 →30 mg and day 5 → 30 mg**.

After completion of the initial dosing, alemtuzumab will be given to all dosing cohorts **during weeks 4, 5 and 6 as the standard 30 mg on MWF schedule** resulting in a total of 13 alemtuzumab treatments.

3.1.1 Dose-Limiting Toxicity for rhIL-15

There are some well-described, clinically inconsequential side effects associated with IL-15 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. Unlike cytopenias noted in patients receiving cytotoxic chemotherapies or alemtuzumab treatment, 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. As such, rh-cytokine treatment resulting in grade 3 or 4 cytopenia, especially lymphopenia, in the absence of signs of infection is not interpreted as a DLT.

DLT will be defined as follows:

Any grade 3 or 4 toxicity possibly, probably or definitely related to the rhIL-15 treatment that occurs during the first 6 weeks 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 neutropenia
    - rhIL-15 will be continued in the event of grade 3 neutropenia 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.
- Grade 3 or 4 anemia that lasts for ≤ 3 days and does not cause hypotension or organ dysfunction. If the anemia last for more than 3 days, it is considered a DLT.

Other exceptions:

- Any event that can clearly be determined to be unrelated to the treatment regimen.

3.1.2 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 completed the DLT evaluation window and an assessment of DLT has been completed.

Patients will receive s.c. rhIL-15 for the initial period Monday-Friday week 1 and 2. They will be assigned to a dose level sequentially based on their order of entry into the study. The starting dose will be 0.5 mcg/kg/dose and in the absence of dose-limiting toxicities dose escalation will proceed to evaluate dose levels of 1.0 and 2.0 mcg/kg/dose. This initial part of the treatment will be 14 days in length and will be followed by 4 weeks (13 treatments) of alemtuzumab. Only toxicities occurring in this 6-week period will be considered in determining dose escalation. Dose-limiting toxicities are defined in Section **3.1.1**. Dose-escalation rules are indicated in the table in Section **3.1.2**. As just noted, the first 6 weeks of rhIL-15 and alemtuzumab treatment will be considered in defining criteria for dose escalation and the maximum tolerated dose (MTD).

Dose escalation will proceed in cohorts of 3–6 patients. The MTD is the dose level at which no more than 1 of up to 6 patients experience DLT during the first 6 weeks of treatment, 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 treatment, he or she will not be evaluable for toxicity and will be replaced in the dose level. An additional 3 patients may be enrolled to compensate for inevaluable patients.

Dose escalation table:

Dose Escalation Schedule	
Dose Level	Dose of IND Agent*

Dose Escalation Schedule	
Level 1	0.5 mcg/kg/dose
Level 2	1 mcg/kg/dose
Level 3	2 mcg/kg/dose
* Doses are stated as exact dose in units (e.g., mg/m^2, mcg/kg, etc.) rather than as a percentage.	

Dose-escalation rules:

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 three (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 six (6) 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 9 patients will be entered at the recommended phase 2 dose.

3.1.2.1 Stopping Rules

Acute ATL patients can have rapidly progressive disease or can have severe disease related metabolic or organ dysfunction that can be exacerbated by treatment with alemtuzumab and fatal events during salvage treatment do occur. If 2 out of the first 15 patients treated in this trial have fatal (grade 5) SAEs regardless of presumed relationship to rhIL-15, entry and all treatment in this protocol will be halted and the data reviewed to better understand the possible contribution of rhIL-15 to these events.

If a patient develops grade ≥3 venous thromboembolism (VTE) during or within four weeks of treatment and while on appropriate VTE prophylaxis, no additional patients will be accrued on the study. Patients already enrolled on the study who are still undergoing treatment will be notified of the event before their next planned alemtuzumab dose and will not receive further doses of alemtuzumab.

3.2 DRUG ADMINISTRATION

rhIL-15 will be given as a subcutaneous (s.c.) injection once daily Monday-Friday following premedication with acetaminophen (preferred) and/or ibuprofen (if reaction despite acetaminophen) as follows:

- 0.5 mcg/kg/dose during weeks 1 and 2 for cohort 1
- 1.0 mcg/kg/dose during weeks 1 and 2 for cohort 2
- 2.0 mcg/kg/dose during weeks 1 and 2 for cohort 3

Patients will receive up to 10 doses of rhIL-15.

NOTE: Refer to **Appendix F** for calculating IL-15 doses. Included in this section is dose calculation for obese patients.

On Day 1 (or before if clinically indicated) of Week 3, patients will be admitted to the NIH Clinical Center inpatient unit for the alemtuzumab infusions. Patients may be discharged from the inpatient unit and treated in the outpatient clinic when their experience with the treatment indicates that the alemtuzumab can be given safely in an outpatient setting. On Day 1 of Week 3, all patients will receive a 3 mg dose of alemtuzumab with premedication (650 mg acetaminophen and 25-50 mg diphenhydramine). If the dose is tolerated without significant toxicity (≤ grade 2 acute, infusion related toxicity and grade 1 or less skin rash) the patient will receive a 10 mg dose on Day 2 of treatment with the same premedication. If this is well tolerated the dose on Day 3 and Day 5 should be 30 mg with the same premedication.

Alemtuzumab will be administered by intravenous (IV) infusion over a minimum of 2 hours and maximum of 12 hours.

NOTE: Refer to Section **14.2.5** Administration procedures: for more detailed instructions about alemtuzumab infusion times.

After patients have completed and tolerated their initiation dosing of alemtuzumab during week 3 of treatment, treatment will be continued on the t.i.w. schedule at 30 mg with premedication as defined above (650 mg acetaminophen and 25-50 mg diphenhydramine) during weeks 4, 5 and 6.

Insertion of percutaneously inserted central catheter (PICC) or other appropriate vascular access device is permitted.

Treatment may be given on inpatient or outpatient basis. Patients may be hospitalized at the NIH Clinical Center Hospital for convenience and/or social reasons. These hospitalizations will not be considered adverse events and therefore will not require expedited reporting to the IRB.

Patients with acute-subtype ATL deemed by the PI or treating investigator to be at high risk for CNS relapse who have not received CNS prophylaxis as part of their prior treatment may receive prophylaxis in the form of cytarabine 40 mg, methotrexate 15 mg, and hydrocortisone 50 mg administered intrathecally (IT) no earlier than Week 3 and every 6-8 weeks thereafter for up to three doses, and may be given on the same day as alemtuzumab. Cytarabine or methotrexate may be omitted in case of prior intolerance or toxicity attributed to either drug. Platelet counts of ≥70,000 /uL need to be confirmed prior to each administration. If CNS disease is detected by cytology or flow cytometry of cerebrospinal fluid taken during IT drug administration, this will be considered disease progression and the patient will be taken off study. These medications (commercial supplies) will be supplied by the Clinical Center with preparation and administration to follow standard clinical guidelines and requirements.

3.3 DOSE MODIFICATIONS

After patients have completed and tolerated their initiation dosing of alemtuzumab during week 3 of treatment, they will continue to receive the standard three times a week (t.i.w.) alemtuzumab dosing (30 mg Monday, Wednesday and Friday) for up to 13 doses in the absence of disease

progression or unacceptable toxicity. In **Table 1** and **Table 2**, and **Figure 10** below describe alemtuzumab dose modifications for toxicities related to alemtuzumab.

Table 1: Dose Modification of Alemtuzumab for non-hematologic toxicity related to alemtuzumab

Grade	Occurrence	Dose	Immediate Action	Resumption of Therapy
1	Any	Any	None	No interruption
2	Any	Any	None	No interruption
3	1st	30 mg	**Hold alemtuzumab until toxicity resolves to Grade ≤ 1 or baseline	Resume alemtuzumab at 10 mg tiw, increase to 30 mg tiw if and when no Grade ≤2 AEs
3	2nd	30 mg	**Hold alemtuzumab until toxicity resolves to Grade ≤ 1 or baseline	Resume alemtuzumab at 10 mg tiw
3	1st	10 mg	**Hold alemtuzumab until toxicity resolves to Grade ≤ 1 or baseline	Resume alemtuzumab at 10 mg tiw
3	2nd	10 mg	Stop alemtuzumab	Stop alemtuzumab
3: allergic reaction	1st	Any	Stop alemtuzumab	Stop alemtuzumab
3: infection	1st	Any	**Hold alemtuzumab until antibiotics stopped	Resume alemtuzumab at 30 mg tiw
	2nd	Any	**Hold alemtuzumab until antibiotics stopped	Resume alemtuzumab at 10 mg tiw
	3rd	Any	Stop alemtuzumab	Stop alemtuzumab
4	Any	Any	Stop alemtuzumab	Stop alemtuzumab
**If alemtuzumab is held >7 days, restart at 3 mg dose and titrate up as in the first week. **Patients with catheter infection or transient bacteremia may restart alemtuzumab sooner if they are clinically stable and surveillance blood cultures on antibiotic treatment are consistently negative.				

Figure 10: Titration and dose modification of alemtuzumab for non-hematologic toxicity related to alemtuzumab

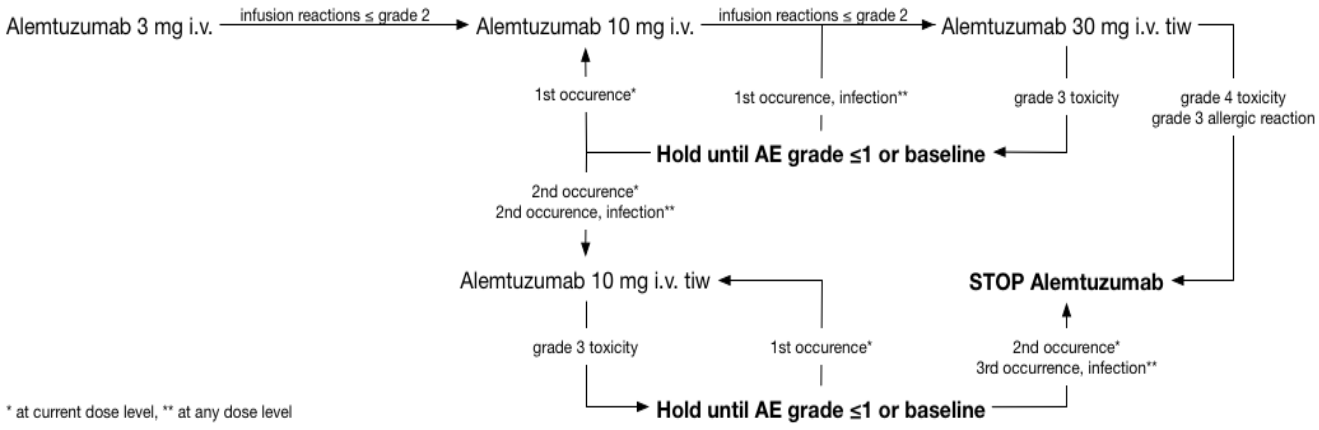

Table 2: Dose Modification of alemtuzumab for hematologic toxicity related to alemtuzumab

Grade	Occurrence	Immediate Action	Resumption of Therapy
1	Any	None	No interruption
2	Any	None	No interruption
3	Any	None	No interruption
4	1st	**Hold therapy until toxicity resolves to Grade ≤ 1 or baseline	Resume alemtuzumab at 30 mg tiw
4	2nd	**Hold therapy until toxicity resolves to Grade ≤ 1 or baseline	Resume alemtuzumab at 10 mg tiw
4	3rd	Stop alemtuzumab	Stop alemtuzumab.
Any grade autoimmune cytopenia	Any	Stop alemtuzumab	Stop alemtuzumab
**If alemtuzumab is held >7 days, restart at 3 mg dose and titrate up as in the first week.			

3.4 CLINICAL MONITORING

3.4.1 Standard Clinical Laboratory Tests or Procedures

Tests may be performed +/- one day in week 1 and +/- 2 days in weeks 2-6. The following tests will be performed on the days listed below during all treatment cycles:

- **Routine chemistry panels** (acute care, hepatic and mineral) and LDH: MWF of each rhIL-15 and alemtuzumab treatment week. Except Week 3, when they will be done on Days 1, 2, 3 and 5.
- **CBC with differential:** MWF of each rhIL-15 and alemtuzumab treatment week. Except Week 3, when they will be done on Days 1, 2, 3 and 5.
- **Serum Lipase and Amylase:** Day 1 of each treatment week
- **CMV PCR; Quantitative, Blood:** Day 1 of each alemtuzumab treatment week
- **Troponin**: Day 1 of each treatment week.
- **Other:** ANA, RF, anti-thyroid antibody and thyroid panel (TSH ± T4, if indicated): Day 1 of weeks 4 and 6.

3.4.2 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 at the end of week 3 and week 6 for comparison to their pretreatment 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 plus alemtuzumab treatment.
- Appropriate patients will have tumor marker (IL-2R alpha) serum levels assessed at the time of their radiographic restaging to gain further insight into the potential efficacy of rhIL-15 plus alemtuzumab treatment.

3.4.3 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.

3.4.4 Clinical Monitoring and Assessments

The following assessments will be performed on the days listed below during all treatment cycles:

- **Vital signs:** Including heart rate, blood pressure, respiration, temperature and oxygen saturation (SO2) by transcutaneous pulse oximeter.
    - Within 2 hours prior to each IL-15 dose and 30-60 minutes after each IL-15 dose
    - During the alemtuzumab treatment, the patient's vital signs (blood pressure, pulse, respirations, and temperature) should be monitored as follows:
        - During the first three doses alemtuzumab (and during subsequent doses until patient's vital signs remain stable during the preceding infusions), every 15 minutes (+/- 5 minutes) for first hour or until stable and then hourly (+/- 15 minutes) until the infusion is discontinued.
        - During subsequent doses (if patient's vital signs were stable during the first three doses): one hour after infusion starts (+/- 15 minutes); then every 4 hours (+/- 30 minutes) for the duration of the infusion, as long as patient is stable and has no signs/symptoms of reaction.
    - All non-treatment inpatient days: every 8 hours (+/- 2 hour); unless inpatient for social reasons, then VS once a day.
    - Outpatient non-treatment days: once per visit.
- **Weight:** Day 1 of each outpatient treatment week and every other day while an inpatient.
- **Interval history and physical examination:** Daily while inpatient, Day 1 (or within 5 days prior to day 1) of each outpatient treatment week and as dictated by adverse events on other outpatient treatment days.
- Documentation of performance status per Study Calendar (Section **3.5**).
- Patients with cutaneous disease will have clinical photography performed to assess their skin disease at the end of week 3 and end of week 6, and a dermatology exam [mSWAT, **Appendix I**] performed at the end of week 6.

3.4.5 Follow-up Testing/Assessments

The following tests and assessments will be performed at every follow-up visit. Follow-up visits will occur every 60 days (± 7 days) for 6 months; then every 90 days (± 14 days) for up to 2 years after finishing treatment; and at the end of study visit (just prior to the patient being taken off study), if feasible:

- Vital signs
- Weight
- History and physical examination, including documentation of performance status
- ANA, RF, anti-thyroid antibody and thyroid panel (TSH ± T4, if indicated)
- Routine chemistry panels (acute care, hepatic and mineral) and LDH
- CBC with differential

- Serum lipase and amylase
- Radiographic evaluation, dermatology exam [mSWAT, **Appendix I**], Clinical Photography and laboratory assessments of disease status.

In addition to the tests listed above, the following tests will be done:

- CBC with differential **monthly** until CD4+ count >200 cells/μL
- FACS (standard TBNK panel) **monthly** until CD4+ cell count >200 cells/μL
- CMV PCR **monthly** for 2 months after alemtuzumab treatment is completed and CD4+ cell count >200 cells/μL

3.5 STUDY CALENDAR

Test/Procedure	**Screening**[1]	**Baseline/Day 1**[2]	**Week 1**		**Week 2**		**Week 3**		**Week 4**		**Week 5**		**Week 6**		**Follow up**[3, 24]	**End of Study**[4]
Days			**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**		
rhIL-15			x	x	x	x										
Alemtuzumab							x[5]		x[5]		x[5]		x[5]			
Intrathecal prophylaxis							x[27]								x[27]	
Pathology confirmed, including CD52 positivity	x															
Flow Cytometry for lymphocyte sets & subsets; and CD52 [10]	x				x[10]					x[10]		x[10]		x[10]	x[10, 24]	
Flow cytometry for lymphocyte sets & subsets (Roederer Lab) [11]		x[11]	x[11]		x[11]					x[11]		x[11]		x[11]		
History & Physical [6]	x	x	x[6]		x[6]		x[6]		x[6]		x[6]		x[6]		x	x
Performance Score	x	x	x[20]		x[20]				x[20]				x[20]		x	x
Vital signs [7]	x	x	x[7]	x[7]	x[7]	x[7]	x[7]	x[7]	x[7]		x[7]		x[7]		x[7]	x[7]
Weight[7]		x	x[7]		x[7]		x[7]		x[7]		x[7]		x[7]		x[7]	x[7]
Height		x														
NIH Advance Directives Form[25]		x														
Imaging Studies (PET, CT, MRI or other as needed to document disease and response)[9]	x	x					x[9]						x[9,15]		x[9]	x[9]
CBC with differential	x	x	x		x		x	x	x		x		x		x[24]	x
Acute Care, Hepatic and Mineral Panels and LDH	x	x	x		x		x	x	x		x		x		x	x
Serum lipase & amylase		x	x[20]		x[20]		x[20]		x[20]		x[20]		x[20]		x	x
Serum pregnancy test[8]	x	x[8]														
Reticulocytes, Glucose-6 phosphate dehydrogenase (G-6PD)		x														
HIV 1&2, HTLV-1&2, Hepatitis B and C	x															

Test/Procedure	**Screening[1]**	**Baseline/Day 1[2]**	**Week 1**		**Week 2**		**Week 3**		**Week 4**		**Week 5**		**Week 6**		**Follow up[3, 24]**	**End of Study[4]**
Days			**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**		
EKG	x															
Pulmonary function tests with DLCO	x															
Thyroid function tests		x							x[21]				x[21]		x	x
Troponin		x	x[20]		x[20]		x[20]		x[20]		x[20]		x[20]			
Urinalysis		x														
ANA, RF and anti-thyroid antibody		x							x[21]				x[21]		x[21]	x[21]
CMV PCR; Quantitative, Blood		x					x[22]		x[22]		x[22]		x[22]		x[24]	
EBV PCR; Quantitative, Blood		x														
HLA typing (A, B, C, DR and DQ)		x														
Reticulocyte Count		x														
Echocardiogram for LVEF	x															
Bone Marrow Biopsy		x														
Clonal T-cell receptor rearrangement by PCR[17]		x														
Two Day and Six Day PBMC proliferation assay[16]		x														
Inflammatory Markers and Cytokine Analysis [12]		x	x[12]				x[12]									
Anti-IL-15 antibodies [13]			x[13]		x[13]				x[13]							
IL-2R alpha and MesoScale Discovery multiplex proinflammatory cytokines [14, 15]		x	x[14]		x[14]		x[14,15]						x[15]		x[15]	
Special FACS and assay for ADCC		x[23]					x[23]	x[23]								
NK cell exhaustion studies		x[26]	x[26]		x[26]											
Skin biopsy if necessary to confirm the diagnosis of ATL or CTCL	x															
MRI scan of the brain and lumbar puncture for patients with neurological symptoms or signs [18]	x															

Test/Procedure	**Screening[1]**	**Baseline/Day 1[2]**	**Week 1**		**Week 2**		**Week 3**		**Week 4**		**Week 5**		**Week 6**		**Follow up[3, 24]**	**End of Study[4]**
Days			**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**	**MWF**	**T+Th**		
Clinical Photography[19]		x					x[9]						x[9]		x[9]	x[9]
Dermatology exam (mSWAT)[19]		x											x		x	x

NOTE: Any other tests should be performed as clinically indicated.

[1] Screening evaluation must be performed within 28 days prior to study enrollment, unless otherwise noted.

[2] Baseline studies must be performed within 28 days prior to initiating treatment unless otherwise noted. It is not necessary to repeat tests that were done for screening if they were done within 28 days prior to initiating treatment. Day 1 studies must be done before that day's dose of study drug. It is not necessary to repeat tests that were done for baseline or screening if they were done within 28 days prior to initiating treatment.

[3] Follow-up visits will occur every 60 days (± 7 days) for 6 months; then every 90 days (± 14 days) for up to 2 years after finishing treatment. 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 for this visit, a request will be made to have a local physician or laboratory collect a CBC w diff and send the results. If this is not possible, patients may be assessed by telephone or email for symptoms.

[4] End of study visit will occur just prior to the patient being taken off study, if feasible.

[5] Alemtuzumab administration is described in Section **3.1**. **Please note**: alemtuzumab administration is different for Week 3: It is administered at 3 mg on Day 1, 10 mg on Day 2, 30 mg on Day 3 and 30 mg on Day 5.

[6] Complete H&P will be done on the Mondays prior to starting rhIL-15 treatment, upon admission to the hospital and for outpatient follow-up visits. A limited or focused interim history and physical exam will be performed daily while inpatient

[7] Vital signs: Including heart rate, blood pressure, respiration, temperature and oxygen saturation (SO2) by transcutaneous pulse oximeter will be measured and recorded as outlined in Section **3.4.4**: Clinical Monitoring and Assessments.

[8] Serum pregnancy test in women of childbearing potential, repeated within 3 days prior to starting the study drug.

[9] At screening, CT scans of neck, chest, abdomen, and pelvis should be performed (with IV and PO contrast, unless patient is allergic or has renal insufficiency); other imaging may be substituted at the discretion of the investigator. Other body areas may be imaged if clinically indicated. MRI of the brain is only required in patients with suspected involvement of CNS. At screening, a FDG-PET/CT torso (extremities to be included if there is confirmed or suspected disease involvement). At baseline one or more of the following studies may be done: CT, MRI, FDG-PET/ CT and

or clinical photography. All patients will have repeat radiologic studies and Clinical Photography to assess marker lesions (measurable) and evaluable lesions (non-measurable) performed at the end of week 3 and end of week 6 for comparison to their pretreatment studies. FDG/ PET to be done at Week 3 and After Cycle 6. Confirmatory scans may need to be done to confirm CR.

[10] One 10 mL lavender-top tube will be drawn for FACS analysis. Samples will be sent to the Immunology Lab, NIH Clinical Center Bldg. 10/ Room 2C410. 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 **5.1.1** for collection times.

[11] Two 10 mL lavender-top tubes will be drawn and sent to Clinical Support Laboratory, Frederick to viably freeze PBMCs. See Section **5.1.1** for collection times.

[12] One 4 mL serum separator tube (SST) will be drawn for Inflammatory Markers and Cytokine Analysis. Samples will be sent to Clinical Support Laboratory, Frederick. See Section **5.1.2** for collection times.

[13] One 4 mL serum separator tube (SST) will be drawn for anti-IL-15 antibodies and sent to Clinical Support Laboratory, Frederick. See Section **5.1.3** for collection times.

[14] One 4 mL serum separator tube (SST) will be drawn for IL-2R alpha and MesoScale Discovery multiplex proinflammatory cytokines of interferon gamma, IL-1 beta, TNF-alpha, IL-12, p70, IL-6, IL-10. Samples will be sent to Clinical Support Laboratory, Frederick. See Section **5.1.4** for collection times.

[15] Patients will also have IL-2R alpha serum levels assessed at the time of their radiographic restaging to gain further insight into the potential efficacy of rhIL-15 plus alemtuzumab treatment.

[16] To be collected from select patients, (this will be determined by physician PI or AI). Send four 10 mL sodium heparin green top tubes to Bonita Bryant (Building 10, Room 3B/35) for processing. The samples for the two-day proliferation assay will be sent to the lab at NCI/Leidos Biomedical Research, Inc.-Frederick, Clinical Support Laboratory for processing and analysis. The six-day proliferation assays will be performed in the Lymphoid Malignancies Branch Lab of Bonnie Bryant. The proliferation analyses are not a CLIA-certified (they are being collected for correlative studies). Every effort should be made to draw and send these tubes to Ms. Bryant by 10 AM.

[17] Send one 10 mL sodium heparin green top tube for clonal T-cell receptor rearrangement by PCR to Dr. Mark Raffeld, Laboratory of Pathology, Building 10, Room 2N110, Phone: (301) 496-1569.

[18] Patients with neurological symptoms or signs will undergo an MRI scan of the brain and lumbar puncture before enrollment.

[19] Patients with cutaneous disease will have clinical photography performed to assess their skin disease at the end of week 3 and end of week 6, and dermatology exam performed to assess the mSWAT score at the end of week 6.

[20] Day 1 of each treatment week.

[21] Day 1 of week 4 and week 6.

[22] Day 1 of each alemtuzumab treatment week.

[23] To be collected from select patients, (this will be determined by physician PI or AI). At Baseline, on Monday of Week 3 before administration of the first dose of alemtuzumab, and on Tuesday of Week 3 just prior to the second dose of alemtuzumab send up to four 10 mL sodium heparin green-top tubes to Sigrid Dubois, Ph.D., Lymphoid Malignancies Branch, in Building 10, Room 4B47, and Phone: (301) 435-4441.

[24] Additional tests to be done during follow-up period:

- CBC with differential **monthly** until CD4+ count >200 cells/μL.
- FACS (standard TBNK panel) **monthly** until CD4+ cell count >200 cells/μL.
- CMV PCR **monthly** for 2 months after alemtuzumab treatment is completed and CD4+ cell count >200 cells/μL.

[25] As indicated in Section **12.3**, all subjects will be offered the opportunity to complete an NIH advance directives form. This should be done preferably 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.

[26] To be collected from select patients, (this will be determined by PI or AI). At Baseline, on Wednesday of Week 1 before IL-15 administration, and on Monday of Week 2 before IL-15 administration, send up to three 10 mL sodium heparin green-top tubes to Clinical Support Laboratory, Frederick, to viably freeze PBMCs. Tubes to be picked up by Bonita Bryant (Building 10, Room 3B/35, Phone (240) 858-3479) or courier.

[27] For patients with acute subtype ATL without prior CNS prophylaxis. Cytarabine 40 mg, methotrexate 15 mg, and hydrocortisone 50 mg administered intrathecally no earlier than Week 3 and every 6-8 weeks thereafter for up to three doses. See Section **3.2** for additional information.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.6.1 Criteria for removal from protocol therapy

Prior to 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. Criteria for removal from protocol therapy:

- Unacceptable adverse event(s), unless it is felt by the Principal Investigator to be in the patient's best interests to remain on study in exceptional circumstances, including:
  - DLT as defined in Section **3.1.1**
  - Persistent (> 14 days) treatment-related grade 2 (non-DLT) adverse events
  - Grade 3 or higher alemtuzumab allergic (infusion) reaction
  - Third occurrence of alemtuzumab related grade 3 infection
  - Third occurrence of alemtuzumab related grade 4 hematologic toxicity
  - Any grade auto-immune cytopenia
  - Grade ≥3 VTE during or within four weeks of protocol treatment while on appropriate VTE prophylaxis
- The detection of anti-IL-15 neutralizing antibody serum titer ≥ 500 ng/mL as described in **Appendix E** prior to subsequent treatment cycles.
- Evidence the patient has developed autoimmunity 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.
- Inability to receive all doses of rhIL-15 treatment during treatment weeks 1 and/or 2 for any reason.
- Positive pregnancy test
- Completion of protocol therapy including a 30-day safety visit
- Disease progression

3.6.2 Off-Study Criteria

- Voluntary withdrawal of consent by the patient to participate in the study.
- Patient noncompliance with treatment and instructions from study team.
- Institution of another anticancer treatment. **NOTE:** patients who undergo consolidative stem cell transplant or cellular therapy after achieving a complete or partial response to protocol treatment will continue to be on study and followed for progression-free survival.
- Any new medical or psychiatric condition that, in the opinion of the PI, precludes the safe continuation of the study.
- Completion of follow-up period
- Death

4 CONCOMITANT MEDICATIONS/MEASURES

All patients will receive the following recommended concomitant pre-medications to lessen the acute side effects associated with alemtuzumab infusions.

4.1 PREMEDICATION FOR ALEMTUZUMAB INFUSIONS

- Diphenhydramine (25 to 50 mg) IV or orally and acetaminophen (650 mg) orally 15 to 90 minutes prior to all the infusions.
- The doses of these medications may be tapered down or additional premedications (ibuprofen or other non-steroidal anti-inflammatory drugs [NSAIDs]) or anti-emetics added as needed to lessen the acute side effects.
- Avoid NSAIDs in patients with a platelet count <50,000/mm^3
- Patients with more severe or significant post treatment side effects may require institution of other appropriate medications (e.g., steroids, epinephrine or meperidine) as needed.

4.2 PREMEDICATION FOR IL-15

- Acetaminophen 650 mg orally 30 to 60 minutes prior to each IL-15 injection as first line and ibuprofen 400 mg or 600 mg orally depending on reactions with acetaminophen as a premed.

4.3 PROPHYLAXIS FOR VIRUS AND/OR PNEUMOCYSTIS

- Patients will receive empiric anti-viral prophylaxis with acyclovir 400 mg twice daily or equivalent for herpetic prophylaxis.
- Patients will receive empiric prophylaxis for *Pneumocystis jiroveci* pneumonia (PJP) with trimethoprim/sulfamethoxazole double strength (DS) one tablet three days each week. The preferred schedule is Monday, Wednesday, Friday. Patients who have G-6PD deficiency, Grade ≥3 neutropenia lasting more than 3 days, and/or allergy to either component may receive inhaled pentamidine 300 mg once a month or other standard treatments.
- PJP and herpes viral prophylaxis will be continued for a minimum of 2 months after completion of the protocol treatment and the CD4+ count is > 200 cells/microL.

4.4 VENOUS THROMBOEMBOLISM (VTE) PROPHYLAXIS

- Patients will be treated with appropriate anticoagulation prophylaxis for VTE, which may include Enoxaparin, Rivaroxaban, heparin or other anticoagulants (see Section **1.2.5** for rationale).
- If a patient develops grade ≥3 VTE during or within four weeks of protocol treatment despite appropriate prophylaxis, study will be stopped and other patients notified per Section **3.1.2.1.**

4.5 PLATELETS AND RBC SUPPORT

- Platelets and RBC support will be given as medically indicated to maintain a hemoglobin > 8gm/dL and to maintain the platelet count at a level of at least 10,000/mm^3 (unless there is evidence of bleeding in which case a platelet count of 50,000/mm^3 will be maintained. All administered blood products will be irradiated and depleted of leukocytes.

4.6 PROHIBITED MEDICATIONS

Patients are prohibited from receiving the following therapies during treatment on this trial:

- Other therapy for the disease under study not specified in this protocol, unless specifically noted as permitted
- Investigational agents other than rhIL-15 and alemtuzumab
- Radiation therapy
  NOTE: Radiation therapy to a symptomatic solitary lesion may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

Patients who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from study treatment. Patients may receive other medications that the investigator deems to be medically necessary.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH AND SPECIAL STUDIES

At the discretion of the PI the following correlative studies may be performed on patients' PBMCs. Prior to administration of IL-15 on day 8 (week 2 day 1), and on day 12 (week 2 day 5), the last day of IL-15 administration, the PBMCs may be evaluated for their capacity to do antibody-dependent cellular cytotoxicity using the laboratory's standard assay. The reactive agents will be both the patients' PBMCs and the PBMCs depleted of CD4 cells using magnetic B column to yield a population of patients' monocytes, NK and B cells. The targets will be both the ATL cell line 43Tb- as well as patients' CD4 T-cells isolated using Miltenyi magnetic beads.

All research samples will have labels affixed to the tubes by the staff person who obtained the samples. The labels will list the patient's name, date of birth, date and time of the blood draw.

5.1.1 Flow-Cytometry

Flow-Cytometry Studies will be done to assess for absolute numbers, percentages of lymphocyte sets, and T-cell subsets.

All FACS samples will be drawn in 10 mL lavender-top tubes and will be performed for the days listed below **during all treatment cycles.** FACS samples should be drawn before the day's dose of study drug. FACS panels will be performed by the Fleisher and Roederer Laboratories for all time points listed. If the Immunology Section Laboratory (Fleisher's Lab) is unable to perform the analysis on the specified days, this assessment maybe omitted or replaced with standard TBNK panel.

- Day 1 Pretreatment:
  - Two 10 mL lavender top tubes drawn for cryopreservation, Clinical Support Laboratory, Building/Room 469/107A, 1050 Boyles Street, Frederick Maryland 21702, telephone number: (301) 846-1707 or (301) 846-1917.
  - One 10 mL lavender top tube will be drawn and sent via routine collection routing to the **Immunology Laboratory of the NIH Clinical Center** 10/ for CD2, CD3, CD4, CD7, CD8, CD14, CD16, CD20, CD25, CD45RA, CD52, CD56, CD57,

IL-2R/IL-15R beta (CD122), CD127 (IL-7R alpha), CD194, CD62L, IL-15R alpha.

- **Day 2:** Two 10 mL lavender top tubes will be drawn (+/- one hour) prior to administration of that day's rhIL-15 injection for FACS analysis and sent to the Clinical Support Laboratory for cryopreservation.
- **Days 8, 26, 33 and 40:** One 10 mL lavender top tube will be drawn with the routine blood draws on days 8 (+ 1 day), 26 (+/- 2 days), 33 (+/- 2 days), and 40 (+/- 2 days). Samples will be sent to the \ Clinical Center Immunology Lab as listed previously. Two 10 mL lavender top tubes will also be drawn for the Roederer Lab (NIAID) at the same time and sent as specified above.

5.1.2 Inflammatory Markers and Cytokine Analysis

- All samples will be drawn in 4 mL SST tubes at the times listed below**.**
  - Week 1, Day 1 pre-therapy and 4 hours (+/- 15 mins), 8 hours (+/- 30 mins), 12 hours (+/- 30 mins) and 24 hours (+/- two hour) after first IL-15 administration
  - Week 3, Day 1 pre-therapy and 4 hours (+/- 15 min), 8 hours (+/- 30 mins), and 24 hours (+/-two hours) after first administration of alemtuzumab.
- Samples will be sent via courier to:

  Clinical Support Laboratory
  Building/Room 469/107A
  1050 Boyles Street
  Frederick Maryland 21702
  Telephone number: (301) 846-1707 or (301) 846-1917)

5.1.3 Anti-IL-15 Antibodies

- All samples will be drawn in 4 mL SST tubes at the times listed below.
  - Before first dose of IL-15
  - Days 12 (+/- 1 day), 26 (+/- 2 days), 33(+/- 2 days), and 40 (+/- 2 days), prior to administration of that day's study drug.
- Samples will be sent via courier to:

  Clinical Support Laboratory
  Building/Room 469/107A
  1050 Boyles Street
  Frederick Maryland 21702
  Telephone number: (301) 846-1707 or (301) 846-1917)

5.1.4 IL-2R alpha and MesoScale Discovery

IL-2R alpha and MesoScale Discovery multiplex proinflammatory cytokines (including interferon gamma, IL-1 beta, TNF-alpha, IL-12, p70, IL-6, IL-10) will be performed.

- All samples will be drawn in 4 mL SST tubes at the times listed below.
  - Before first dose of IL-15
  - After last dose of s.c. IL-15 (+/- 30 mins)
  - Week 3, Day 1 pre-therapy and 4 hours (+/- 15 mins), 8 hours (+/- 30 mins), and 24

hours (+/-two hour) after first administration of alemtuzumab

- Samples will be sent via courier to:

  Clinical Support Laboratory
  Building/Room 469/107A
  1050 Boyles Street
  Frederick Maryland 21702
  Telephone number: (301) 846-1707 or (301) 846-1917

5.1.5 Special FACS analysis

Special FACS analyses will be performed, including of NK cell and ATL/T-PLL/CTCL populations.

- Samples to be drawn from select patients, (to be determined by Physician PI or AI): up to four 10 mL sodium heparin green-top tubes at the times listed below.
  - At baseline
  - Week 3, Day 1 before the first administration of alemtuzumab
  - Week 3, Day 2 just prior to the second administration of alemtuzumab
- Samples will be sent to:

  Sigrid Dubois, Ph.D.
  Lymphoid Malignancies Branch
  Building 10, Room 4B47
  Phone: (301) 435-4441

5.1.6 NK cell exhaustion analysis

PBMCs will be viably frozen for batch processing and analysis for immunophenotypic (via FACS) and functional (via cytotoxicity assays) evidence of NK cell exhaustion.

- Samples to be drawn from select patients (to be determined by Physician PI or AI): up to three 10 mL sodium heparin green-top tubes at the times listed below.
  - At baseline
  - Week 1, Day 3 before the third administration of IL-15
  - Week 2, Day 1 before the sixth administration of IL-15
- Samples will be picked up by:

  Bonita Bryant
  Building 10/Room 3B33
  Phone: (240) 858-3479
  (or designated LYMB staff)
- Samples will be sent via courier to:

  Clinical Support Laboratory
  Building/Room 469/107A
  1050 Boyles Street
  Frederick Maryland 21702
  Telephone number: (301) 846-1707 or (301) 846-1917)

5.1.7 Adult Patients: Blood Drawing Limits for Research Purposes

The amount of blood that may be drawn from adult patients (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

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 appropriate approvals and/or agreements, if required.

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 below. 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.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

5.2.1 Procedures for stored serum specimens in the Clinical Support Laboratory, Leidos Biomedical Research, Inc.:

- The Clinical Support Laboratory, Leidos Biomedical Research, Inc.-Frederick, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is CLIA certified for anti-IL 15 and certain cytokine measurements, 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, 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 (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 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. At a minimum, the lab needs confirmation that one has been executed or an exception was granted from an office authorized to make such exceptions, e.g., NCI Technical Transfer Center. 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 approvals and/or agreements, if required, prior to requesting the laboratory to ship samples outside of the NIH.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

6.1.1 Summary

The PI will be responsible for overseeing entry of data into an in-house password protected

electronic system (C3D) 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 AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed for at least 30 days after the last study drug administration and until return to baseline or stabilization of event.

Document AEs from the first study intervention through 30 days after the last study intervention was administered. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

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, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.2 Data Collection/Recording Exceptions

6.1.2.1 Abnormal Laboratory Values

An abnormal laboratory value will be considered an AE 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.

6.1.2.2 Data Entry Exceptions

The following data will not be entered into C3D:

- Results of History and Physical (H&P) exams.
- Vital Signs (except Baseline Weight and Height which will be entered).

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository.

_X_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_X_ Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

_X_ An NIH-funded or approved public repository. Insert name or names: ClinicalTrials.gov.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

This is not applicable to this study at this time as there are no planned genomic studies.

6.3 RESPONSE CRITERIA

Response and progression 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)(87). 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. Separately, patients with cutaneous involvement will have their skin disease evaluated by a dermatologist using the mSWAT tool [**Appendix I**]. Leukemic cells (Sezary or T-PLL) as percentage of PBMCs will also be assessed and reported separately.

6.3.1 Definitions

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

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have completed the 6 weeks 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.

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 completed the 6 weeks 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.

6.3.2 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:

- By chest x-ray: ≥ 20 mm
- By CT scan:
  - Scan slice thickness of 5 mm or under: as ≥10 mm
  - Scan slice thickness of >5 mm: double the slice thickness

- With calipers on clinical exam: ≥10 mm

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

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.

6.3.3 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(88-90). 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(91).

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:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- 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.
- 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.

6.3.4 Response Criteria

In addition to the standard RECIST response criteria below, the following will be also applicable to evaluation of response for skin lesions for this study:

Target Lesions:

- Complete Response (CR): Disappearance of skin lesions per mSWAT/Global Response Score and/or any circulating leukemic cells must also be documented, if initially present.
- Partial Response (PR): At least a 50% decrease in ATL skin lesions (per mSWAT/Global Response Score) and/or in the absolute number of circulating leukemic cells, if initially present.

Non-Target Lesions:

- Complete Response (CR): Disappearance of skin lesions per mSWAT/Global Response Score and/or any circulating leukemic cells must also be documented, if initially present.

6.3.4.1 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 of 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 of diameters while on study.

6.3.4.2 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).

6.3.4.3 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**

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
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. ** 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		

6.3.5 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.

6.3.6 Progression Free Survival

Progression Free Survival will be measured from the date of protocol consent until death or progressive disease occurs.

6.4 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 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found **here**.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/ IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found **here**. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to definitions provided in Policy 801: Reporting Research Events found **here**.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED 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 or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

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.

8 SPONSOR SAFETY REPORTING

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2).

8.1.2 Serious Adverse Event (SAE)

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 event (see **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- 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.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment Of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1.1**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at:

https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.**8.3**.

8.4 SAFETY/OTHER REPORTING CRITERIA TO THE PHARMACEUTICAL MANUFACTURER (CTEP)

The NCI Cancer Therapy Evaluation Program (CTEP) is providing clinical grade recombinant human IL-15 for this study. Because the CTEP is responsible for the Clinical Material and Confidential Information which it develops, CTEP must ensure that the Clinical Material and Confidential Information are used, communicated and reproduced appropriately and completely. The PI agrees to use the Clinical Material in accordance with all Federal laws and regulations that govern the use of investigational agents in clinical trials.

The following will be provided to CTEP during the course of the clinical study:

1. Initial FDA submission/approval, including: FDA-submitted protocol document; any FDA comments regarding the protocol and IND submission, including correspondence regarding the IND submission safe-to-proceed notice; and, a copy of the FDA acknowledgement of the IND submission(s) stating the IND number, sponsor, title and date of submission.
2. Documentation of initial IRB approval of the FDA-submitted protocol document and annual continuing IRB review approvals.
3. All significant protocol amendments, including changes in study size, eligibility criteria, study design and end points.
4. Notification of any changes in protocol status or other significant events related to the Protocol.
5. Notification of any changes in IND status.
6. Copies of any FDA communications.
7. Copies of IND Annual Reports.
8. All IND Safety Reports submitted to the FDA per 21 CFR 312.32. Copies of all IND Safety Reports submitted to the FDA should be forwarded electronically to CTEPSupportAE@tech-res.com (please provide protocol number in subject line).
9. Any abstracts, manuscripts, and publications.

Additional safety and efficacy data may also be requested by CTEP to facilitate the development of the Clinical Material across CTEP supported trials.

8.5 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here:
https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.5.1 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 no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later

than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (**8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.5.2 Paternal Exposure

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

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 four (4) months after the last dose should, if possible, be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING

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 and secondary study endpoints; adherence to the protocol, regulations, ICH E6 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.

10 STATISTICAL CONSIDERATIONS

The primary objective of this trial is to determine the safety, toxicity profile, DLT and MTD of rhIL-15 administered at escalating doses s.c. in combination with alemtuzumab. There are three planned dose levels (0.5, 1, and 2 mcg/kg/dose s.c.) and one dosing strategy (weeks 1 and 2 Monday-Friday). In week three all patients will receive alemtuzumab: 3 mg on day 1, 10 mg on day 2, 30 mg on day 3 and 30 mg on Day 5 of the study. The therapy with alemtuzumab will be continued on weeks 4, 5 and 6 on a 3 x weekly schedule at 30 mg for a total of 4 weeks (13 treatments) of alemtuzumab.

The determination of MTD follows the 3+3 design in which 3 to 6 patients will be enrolled in each combination of dose level. In the initial cohort (Cohort 1), at least 3 patients will be enrolled to receive 0.5 mcg/kg/dose of rhIL-15. In Cohort 2, patients will be enrolled to receive 1 mcg/kg/dose of rhIL-15; and in Cohort 3 patients will receive 2 mcg/kg/dose of IL-15.

The MTD will be based on the assessment of DLT during the first 6 weeks 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 (≥2/3 or ≥2/6 patients) having DLT. If a patient did not experience DLT and did not finish the 6 weeks of therapy, this patient 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 be treated at this dose to better characterize the clinical activity of rhIL-15 in this patient population. Accordingly, if all dose levels are evaluated (6 patients per dose level with 9 total patients at the MTD or maximum administered dose) a maximum of 21 evaluable patients will be enrolled. Similarly, if all dose levels are evaluated, the minimum number of evaluable patients required will be 15. An additional 9 patients may be enrolled to compensate for inevaluable patients; thus, bringing the maximum accrual ceiling to 30.

It is expected that accrual can be completed in 60 months.

Using this dose escalation scheme, the probability of escalating to the next dose level, based on the true rate of DLT at the current dose, 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

The secondary objectives are first to evaluate the potential antitumor activity of the combination of rhIL-15 and alemtuzumab assessing the clinical response rate and time to progression, Another secondary objective is to characterize the biological effects of rhIL-15 administered with alemtuzumab by FACS analysis to define the percentages and absolute numbers of circulating lymphocytes (T and NK cells) and the T-cell subsets naïve, central and effector memory subsets (based on expression of CD52, CD56, CD4, CD8, CD45RO, CD45RA, CD28, CD95, CD194, and CCR7) by flow cytometry. Finally, a secondary objective is to define the effect of the combination on the plasma levels of proinflammatory cytokines using Mesoscale analysis.

The secondary objectives of the study include characterize the biological effects of rhIL-15 administered with alemtuzumab on the percentages and absolute number of circulating lymphocytes (T and NK cells) and the T-cell subsets naïve, central and effector memory subsets (based on expression of CD52, CD56, CD4, CD8, CD45 RO, CD45RA, CD28, CD95, CCR7, and CD62L) by flow cytometry, and plasma levels of proinflammatory cytokines. Summary statistics will be reported for these measures and considered exploratory.

The accrual ceiling of 24 participants was determined to include the sample size needed for statistical analysis of the safety, toxicity and maximum tolerated dose in this Phase I trial. It provides for 3 inevaluable participants as well as for an expanded cohort of 9 participants at the maximum tolerated dose.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

There is an MTA with Division of Cancer Treatment and Diagnosis (DCTD) for the IL-15 (#15-2-0064).

12 HUMAN SUBJECTS PROTECTIONS

12.1 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 in Section **2.1**. 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 and/or breastfeeding women will be excluded from participation in this trial. Since s.c. 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 are much less likely to have benefit from this immune-based therapy and 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.

12.2 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 may be the object of a separate protocol once toxicity and a dose range with biological activity are defined in adults.

12.3 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 **12.4**), 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 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.

12.4 Evaluation of Benefits and Risks/Discomforts

The discomforts of the 10-day s.c. injections 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 7 and appropriate care provided.

The intent of the study is to evaluate the toxicity of rhIL-15 plus alemtuzumab in this Phase I trial and to find a range of doses and dosing schedules where it has an effect on humans (biological activity). Recently there has been new evidence to suggest that rhIL-15 increases the number and state of activation of NK cells and monocytes. These findings lend additional weight to the notion that the administration of rhIL-15 might be associated with an increase in the ADCC mediated by alemtuzumab that in turn is associated with a longer duration of partial or complete responses of the patient's tumor.

12.5 Risks/Benefits Analysis

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

This risk/benefit analysis applies to all populations of patients eligible for inclusion in this study, including adults able to consent at enrollment as well as those who may become unable to consent during participation.

12.5.1 Risks Related to Imaging

CT, PET and MRI scans often use a contrast agent. There is a small risk of having a reaction to the contrast and most often include nausea, pain in the vein where the contrast is given, headache, metallic and/ or bitter taste in the mouth and a warm, flushing feeling. Rarely, some people have more severe allergic reactions to the contrast which may include skins rashes, shortness of breath, wheezing or low blood pressure.

MRI scans may also involve the use of gadolinium contrast. Although most of the gadolinium is excreted in urine, FDA recently issued a safety alert that indicates small amounts of gadolinium may remain in the body for months to years. The effects of the retained gadolinium are not clear. At this time, retained gadolinium has not been linked to health risks in people whose kidneys

work well. People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis" which has resulted in a very small number of deaths. A blood test of kidney function may be done within the month before an MRI scan with gadolinium contrast.

12.5.2 Risks from Radiation Exposure

The procedures for performing the CT and FDG PET/CT scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. In summary, subjects may receive an annual maximum radiation exposure from up to eight (8) CT scans of the neck, chest, abdomen, and pelvis, and four (4) FDG PET/CT scans for this study. NOTE: CT is expected to be the imaging of choice; PET/CT may be done concurrently in some instances and is optionally accounted for at a lesser number.

The total additional radiation dose for research purposes will be approximately 15 rem.

12.6 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided to the participant or consent designee(s) (e.g., the legally authorized representative [LAR] if participant is an adult unable to consent in cases of reconsent) for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping

- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe the site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical

records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI CCR: NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

This study is being conducted under a CCR-held IND: IND #129263.

14.1 RECOMBINANT HUMAN INTERLEUKIN-15 (RHIL-15) (NSC# 745101)

14.1.1 Source

rhIL-15 is an investigational agent supplied to the investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. Study-specific supply for this study is designated; contact Dr. Miljkovic or Dr. Waldmann with questions.

14.1.2 Drug Summary Information

14.1.2.1 Chemical Name or Amino Acid Sequence

The 115 amino acid coding sequence of the pET28b/IL-15 cistron is as follows:

MNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDA
SIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

14.1.2.2 Other Names

Recombinant Human Interleukin -15; Recombinant Human IL-15; rhIL-15

14.1.2.3 Classification

Recombinant human interleukin-15 (rhIL-15) is a cytokine of the 4-alpha helix bundle family of cytokines whose mature form consists of 115 amino acids. It has two cystine disulfide cross linkages at positions Cys 42-Cys 88 and Cys 35-Cys 85.

14.1.2.4 Molecular Weight (M.W.)
12,898.8 Daltons

14.1.2.5 Mode of Action
IL-15 interacts with a private receptor subunit IL- 15R alpha as well as the IL-2/IL-15R beta chain shared with IL-2 and the common gamma chain shared with IL-2, IL-4, IL-7, IL-9 and IL-21. IL-15 shares a number of biological activities with IL-2, including stimulation of the proliferation of activated CD4+, CD8+ as well as gamma-delta subsets of T cells. IL-15 also stimulates the proliferation of NK cells and acts as a co-stimulator with IL-12 to facilitate the production of Interferon-gamma and TNF-alpha.

14.1.3 How Supplied

IL-15 is manufactured by the Biopharmaceutical Development Program (BDP) and distributed by the Pharmaceutical Management Branch (PMB) ant CTEP. IL-15 is supplied as a sterile, frozen liquid product in single use vials containing no preservatives. Currently, IL-15 is supplied as 147 mcg / 0.3 mL (490 mcg/mL) in a 3 mL glass vial. The IL-15 is formulated in 25 mM sodium phosphate containing 0.5 M sodium chloride at a pH of 7.4.

NOTE: IL-15 vial content may vary between lots and protocols. Use caution and consult the protocol document for specific preparation instructions when preparing each dose.

14.1.4 Preparation

Vials of frozen IL-15 should be thawed at ambient room temperature. Upon thawing, the solution should be clear and colorless with no evidence of particulates or foreign matter. The infusion solutions should be mixed in a PVC bag.

14.1.5 Storage

IL-15 vials should be stores at or below (-70°C).

14.1.6 Stability

14.1.6.1 Vials
Stability studies of the intact vials are ongoing.

14.1.6.2 Prepared Infusion
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 2-8°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., the drug manufacturer.

14.1.7 Administration

rhIL-15 drug product for subcutaneous administration should be prepared as described in **Appendix F**.

The needles used for study treatment administration should be suitable for subcutaneous injection.

The rhIL-15 will be administered at the assigned dose (0.5 mcg/kg/dose, 1 mcg/kg/dose or 2 mcg/kg/dose) as a subcutaneous injection once daily (Mon thru Fri) during weeks 1 and 2 for a total of 10 doses.

14.1.8 Toxicity

The Comprehensive Adverse Event and Potential Risks List (CAEPRs) for Recombinant Human IL-15 provides a single list of reported and/or potential adverse events (AE) associated with the agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for Recombinant Human IL-15.

Version 1.3, January 2, 2019*

Adverse Events with Possible Relationship to Recombinant Human IL-15 (CTCAE 5.0 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	

Adverse Events with Possible Relationship to Recombinant Human IL-15 (CTCAE 5.0 Term)	Specific Protocol Exceptions to Expedited Reporting (SPEER)
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)***

*This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail

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; Visceral arterial ischemia

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; 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; Infusion related reaction

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

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.

14.1.9 CTEP Information

14.1.9.1 Agent Ordering and Agent Accountability

NOTE: See study-specific study drug supply notation in Section **14.1.1**.

NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, 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 participating investigator at that institution.

The CTEP Pharmaceutical Management Branch (PMB) will provide direction as to when sites can order PMB-supplied agents.

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, a “current” password, and active person registration status. 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.

14.1.9.1.1 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.

14.1.9.2 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an “active” account status, a “current” password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

14.1.9.3 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.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)

14.2 ALEMTUZUMAB (CAMPATH-1H)

Alemtuzumab causes the lysis of lymphocytes by fixing to CD52, a highly expressed, non-modulating antigen on the surface of lymphocytes. It mediates the lysis of lymphocytes via complement and antibody-dependent, cell-mediated cytotoxicity mechanisms.

14.2.1 Source

Available through the Campath Distribution Program (The Sanofi Foundation for North America 1-877-422-6728). Vials are provided through this program upon completion of a patient specific request form. Prior to submission of a drug request the patient must provide authorization for the release of medical information (NIH-527). Refer to the Pharmacy Department or Clinical Pharmacy Specialist for additional details on drug procurement.

14.2.2 Toxicity

The majority of adverse events seen in trials have been administration related and of short duration. Serious adverse events, some of which were fatal, have been observed in association with treatment of alemtuzumab.

Please refer to the package insert for a complete listing of all toxicities.

Infusional reactions occur in most patients. They commonly consist of rigors, fever, headache, nausea, vomiting and diarrhea, rash, pruritus, dyspnea and hypotension. Acute infusional reaction may also include chills, abdominal and back pain, bronchospasm, angioedema, tachyarrhythmia etc. These reactions are most prominent during the first dose of alemtuzumab administration and improve with subsequent treatments. To reduce the frequency and severity of the first dose reaction, a step-up dose escalation schedule and proper premedication should be used.

Myelosuppression: anemia, neutropenia, thrombocytopenia, prolonged and profound lymphopenia.

Infections: common bacterial (pneumonia and sepsis) or opportunistic infections (e.g. Pneumocystis jiroveci pneumonia, oral candidiasis, herpes zoster, CMV reactivation, Cryptococcus).

Reported adverse events by organ systems:

- **Body as a whole:** Allergic reaction, rigors, fever, chills, headache, back and abdominal pain, infection and fatigue.
- **Cardiovascular:** Hypertension, hypotension, tachycardia, atrial arrhythmia, ventricular tachycardia, angina, myocardial infarction and peripheral vasoconstriction.
- **Digestive:** Anorexia, nausea, vomiting, diarrhea, constipation, dyspepsia, liver function abnormality.
- **Hematological:** Neutropenia, lymphopenia, thrombocytopenia, anemia, DIC, hemolysis, eosinophil disorder, bleeding (GI, gum, ecchymosis).
- **Musculoskeletal:** Myalgia, arthritis, bone pain, hypotonia, tremor.
- **Metabolic and Nutritional:** Tumor lysis syndrome, acidosis.
- **Nervous System:** Dizziness, confusion, somnolence, peripheral neuropathy, cerebral hemorrhage, speech disorder, mental status changes, paresthesia, syncope, depression, aphasia.
- **Pulmonary:** Bronchospasm, cough, pleural effusion, pulmonary edema, interstitial pneumonitis.
- **Skin/Subcutaneous:** Angioedema, facial flushing, diaphoresis, pruritus, rash, urticaria, injection site reaction (subcutaneous route).
- **Urogenital:** Hematuria, oliguria, polyuria, urinary retention, urinary tract infection, impotence.
- **Aural:** Tinnitus, hearing loss.

14.2.3 Formulation and preparation

Alemtuzumab is supplied as a clear glass vial containing 30 mg alemtuzumab in 1 mL of solution (8.0 mg sodium chloride, 1.44 mg dibasic sodium phosphate, 0.2 mg potassium chloride, 0.2 mg monobasic potassium phosphate, 0.1 mg polysorbate 80, and 0.187 mg disodium edentate dehydrate). No preservatives are added. Each carton contains three alemtuzumab vials (NDC 50419-357-03) or one alemtuzumab vial (NDC 50419-357-01).

14.2.4 Stability and storage

Vials of alemtuzumab should be stored at a temperature of 2-8°C and protected from light. The desired dose of 30 mg. should be drawn up into a syringe from the ampoule and further diluted in 250 ml of 0.9% sodium chloride. The vial contains no preservatives and is intended for single use only; the vial should be discarded with any unused portion after 6 hours. An internal NIH Pharmacy (Pharmaceutical Development Section) conducted study demonstrated 24-hour stability of alemtuzumab when diluted in 0.9% sodium chloride to a concentration range of 6.67 mcg/mL to 120 mcg/mL at room temperature (Goldspiel JT, et.al. Stability of alemtuzumab solutions at room temperature. Am J Health-Syst Pharm, 2013; 70:439-442). Alemtuzumab solutions prepared in the concentration range described above may be stored at room temperature (15-30°C) for up to 24 hours. Alemtuzumab solutions should be protected from light.

14.2.5 Administration procedures

Alemtuzumab will be administered by intravenous (IV) infusion over a minimum of 2 hours and maximum of 12 hours. Do not give by IV push or bolus. Note: To reduce the severity of first

infusional reactions, alemtuzumab should be started at a low dose (3 mg/d) and increased gradually to the target therapeutic dose. Patients should be pre-medicated with 25 to 50 mg of diphenhydramine and 650 mg of acetaminophen 15 to 90 minutes prior to all infusions.

Medications readily available for the emergency management of anaphylactic reactions should include: epinephrine (1:1000, 1 mg/mL) for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment.

14.2.6 Incompatibilities

None known

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16 APPENDICES

16.1 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.

16.2 APPENDIX B: CHARACTERISTICS OF PATIENTS WITH THE VARIOUS STAGES OF ATL

ATL has been subdivided into 4 categories:

1. Smoldering type:
   - 5 percent or more abnormal T lymphocytes in the peripheral blood in association with a normal lymphocyte level (< 4 x 10^9/L)
   - Lack of hypercalcemia
   - Lactic dehydrogenase (LDH) values no greater than 1.5 x the upper limit of normal
   - No lymphadenopathy or organ involvement other than skin and pulmonary lesions
   - Patients with ATL demonstrable on skin biopsy did not have to manifest 5 percent abnormal cells in peripheral blood
2. Chronic type:
   - Absolute lymphocytosis (4 x 10^9/L or more) with T-cell lymphocytosis more than 3.5 x 10^9/L
   - LDH values up to twice the upper limit of normal
   - No hypercalcemia or involvement of the central nervous system, bone or gastrointestinal tract or manifestations of associated ascites or pulmonary effusions
3. Lymphoma type:
   - No lymphocytosis
   - 1 percent or less abnormal T cells in the circulation, in conjunction with histologically proven malignant lymphadenopathy
4. Acute type:
   - Includes the remaining ATL patients who usually have leukemic manifestations and tumor lesions

16.3 APPENDIX C: IMMUNE BASED STUDIES

Flow Cytometry:

(**Note:** CD52 must be performed)

Tube	FITC	PE	PerCP	CD52	PE-Cy7	APC	APC-efluor 780
1	CD14						CD45 Ungated
2	IgG	IgG	IgG		IgG	IgG	CD45
3	CD45RA	CD62L	CD4		CD3	CD8	CD45
4	CD7	CD25	CD4		CD3	CD8	CD45
5	CD3/CD16+CD56		CD20				CD45
6	CD2	IL15Rα	CD4	CD52	CD3		CD45
7	CD57	MIKβ3	CD127		CD3	CD4	CD45
	Initial Study Only						
8	CD3	CD25	CD194(CCR4)			CD4	CD45

- 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 Department of Laboratory Medicine, NIH Clinical Center.
- Special flow cytometry also performed by Dr. Mario Roederer, NIAID in the Vaccine Research Center, Building 40, Room 5044.
- Assay of rhIL-15 by R&D Systems Quantikine ELISA Kit (Catalog # D1500) to define concentration of IL-1t in pharmacokinetic assay.
- Assay for antibodies to infused IL-15 (see **Appendix E**).

16.4 APPENDIX D: *EX VIVO* PBMC PROLIFERATION ASSAYS

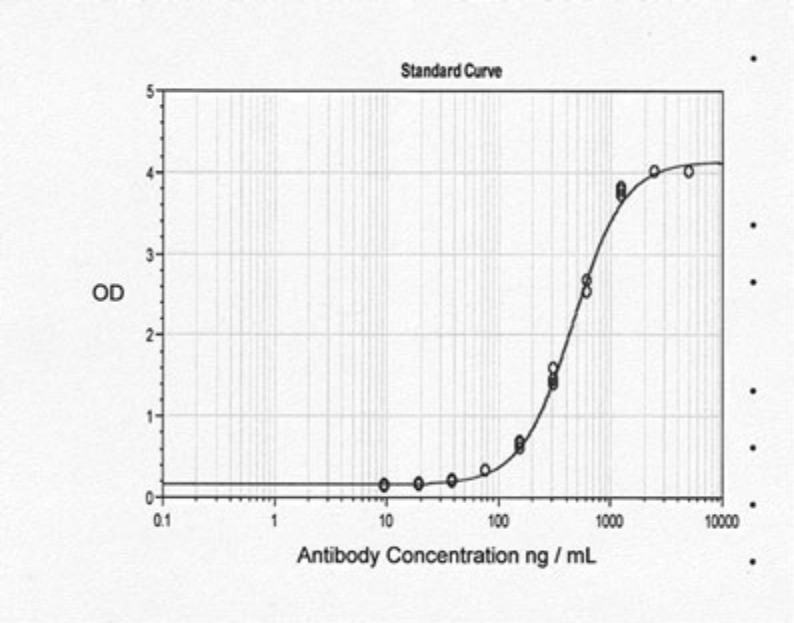
16.4.1 Appendix DA: Two Day *Ex Vivo* Blood Proliferation Assays

- Two day *ex vivo* blood proliferation assays are to be setup Monday through Wednesday and performed on ATL patient peripheral blood mononuclear cells (PBMCs) isolated by Ficoll-Hypaque density gradient centrifugation.
- The PBMCs are to be washed with 1X phosphate-buffered saline (PBS) three (3) times at 1100 rpms for 10 minutes and then resuspended in RPMI 1640 media, supplemented with 10% FBS at a concentration of 1 x 10^6 cells/mL.
- The 100 µL aliquots of the cell suspensions are to be seeded in 96 well-microtiter plates in three (3) sets of triplicates.
- 100 µL of IL-15 is to be added to each well of the first set of triplicates at a final concentration of 50 ng/mL.
- 100 µL of IL-15 is to be added to each well of the second set of triplicates at a final concentration of 5 ng/mL.
- 100 µL of RPMI 1640 with 10% FBS is to be added to each well of the third set of triplicates.
- The plate is to be cultured for two (2) days at 37^{o}C in 5% CO_2.
- The plate is to be pulsed with 1 µCi 3H thymidine 6 hours before harvesting.
- If the additions of either 5 or 50 ng/mL of IL-15 causes both a four-fold increase of the thymidine counts compared to the patient's control value and represent at least 10,000 cpm/minute increase from the controls value, the patient's ATL cells will be deemed to proliferate in response to IL-15.

16.4.2 Appendix DB: Six Day *Ex Vivo* Blood Proliferation Assays

- **Six day** *ex vivo* blood proliferation assays are to be setup and performed Monday through Wednesday on ATL patient peripheral blood mononuclear cells (PBMCs) isolated by Ficoll-Hypaque density gradient centrifugation.
- The PBMCs are to be washed with 1X phosphate-buffered saline (PBS) three (3) times at 1100 rpms for 10 minutes and then resuspended in RPMI 1640 media, supplemented with 10% FBS at a concentration of 1 x 10^6 cells/mL.
- The 100 µL aliquots of the cell suspensions are to be seeded in 96 well-microtiter plates in three (3) sets of triplicate.
- 100 µL of IL-15 is to be added to each well of the first set of triplicates at a final concentration of 50 ng/mL.
- 100 µL of IL-15 is to be added to each well of the second set of triplicates at a final concentration of 5 ng/mL.
- 100 µL of RPMI 1640 with 10% FBS is to be added to each well of the third set of triplicates.
- The plate is to be cultured for two (2) days at 37^{o}C in 5% CO_2.
- The plate is to be pulsed with 1 µCi 3H thymidine 6 hours before harvesting.
- If the additions of either 5 or 50 ng/mL of IL-15 causes both a four-fold increase of the thymidine counts compared to the patient's control value and represent at least 10,000 cpm/minute increase from the controls value, the patient's ATL cells will be deemed to proliferate in response to IL-15

16.5 APPENDIX E: 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.

16.6 APPENDIX F: IL-15 DILUTION INSTRUCTIONS

Drug is provided by Dr. Thomas Waldmann, phone: 240-760-6091 as 500 mcg/mL, 1.0 mL/vial for single dose use and will be stored in minus 70°C freezer.

All dose preparations will be performed in a laminar flow hood in compliance with all legal requirements and in accordance with guidelines of recognized organizations.

D5W will be used for the dilutions listed below. Human Serum Albumin (HSA) is not needed for these concentrations of IL-15.

Please note: The dosing examples listed below are for the 500 mcg/mL vial size and dilution ONLY. The following dosing chart may be used as a reference, but doses should always be re-calculated at the time of preparation. In the future, different concentrations of IL-15 may be available and doses and dilutions will need to be recalculated.

Cohort 1 (0.5 mcg/kg)

To prepare an IL-15 dose for Cohort 1 (0.5 mcg/kg):
Prepare a 1:3 dilution of IL-15.

1. Thaw vial(s) of IL-15, **500 mcg/mL** at room temperature.
2. Add 0.75 mL diluent for IL-15 (5% dextrose) to sterile, empty 10 mL glass vial
3. Add 0.25 mL IL-15 to the vial
4. Yield 125 mcg IL-15/mL
5. Using a 27-gauge needle, slowly draw up the required dose in a 1 mL syringe. Doses should be rounded to the nearest 0.01 mL.
6. Aspirate all fluid from the needle and hub into the syringe. Remove the needle and cap the syringe with a sterile syringe cap.
7. Label the syringe with a 4-hour expiration.

Cohort 1: Subject weight = _____ kg X 0.5 mcg/kg = **Subjects dose = _____mcg**

Subjects dose = _____mcg ÷ 125 mcg/mL = _____mL of rhIL-15

Patient's weight	0.5 mcg/kg (125 mcg/mL dilution)
60 kg	0.24 mL
75 kg	0.30 mL
90 kg	0.36 mL
105 kg	0.42 mL

Dose calculation for obese patients:

Defined as a body mass index (BMI) >30. A corrected body weight will be used to calculate the dose of IL-15 the patient will receive. It will be calculated using the patient's actual weight and a body mass index at the upper limit of normal (BMI = 30). The corrected weight will be calculated from the BMI formula: BMI = weight (in kg)/height (in m squared) or corrected weight (in kg) = 30 x height (in m squared).

Cohort 2 (1 mcg/kg) and Cohort 3 (2 mcg/kg):

To prepare an IL-15 dose for Cohorts 2 (1 mcg/kg) and 3 (2 mcg/kg):

1. Thaw vial(s) of IL-15, 500 mcg/mL at room temperature.
2. Using a 27-gauge needle, slowly draw up the required dose in a 1 mL syringe. Doses should be rounded to the nearest 0.01 mL.
3. Aspirate all fluid from the needle and hub into the syringe. Remove the needle and cap the syringe with a sterile syringe cap.
4. Label the syringe with a 4-hour expiration.

Cohort 2: Subject weight = _____ kg X 1 mcg/kg = **Subjects dose = _____mcg**

Cohort 3: Subject weight = _____ kg X 2 mcg/kg **= Subjects dose = _____mcg**

Subjects dose = _____mcg ÷ 500 mcg/mL = _____mL of rhIL-15

	1 mcg/kg (undiluted 500 mcg/mL)	2 mcg/kg (undiluted 500 mcg/mL)	
60 kg	0.12 mL	0.24 mL	
75 kg	0.15 mL	0.30 mL	
90 kg	0.18 mL	0.36 mL	
105 kg	0.21 mL	0.42 mL	

Dose calculation for obese patients:
Defined as a body mass index (BMI) >30. A corrected body weight will be used to calculate the dose of IL-15 the patient will receive. It will be calculated using the patient's actual weight and a body mass index at the upper limit of normal (BMI = 30). The corrected weight will be calculated from the BMI formula: BMI = weight (in kg)/height (in m squared) or corrected weight (in kg) = 30 x height (in m squared).

16.7 APPENDIX G: TABLES AND FIGURES FROM ALEMTUZUMAB THERAPY OF ATL

Table 1: Patient Demographics

Category	**Characteristics**
Sex	19 Women 10 Men
Age	Median: 48 years (range 24-76)
Race	African-Caribbean: 25 African-American: 3 Japanese: 1
ATL Classification	Lymphoma: 11 Acute Leukemia: 15 Chronic Leukemia: 3
Karnofsky Performance Status	Median: 89.4% (range 80-90%)

Table 2: Prior Therapies Administered to Patients

Prior Treatment	**Number of Cycles**
CHOP	15
Zidovudine and Interferon-alpha	1
Daclizumab	3
Siplizumab	4
Palliative radiation therapy	1
Stable-dose of corticosteroid	6

Table 3: Response of ATL Patients to Alemtuzumab

ATL Classification	Response	Response Duration (Days)	Response Duration (Weeks)
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PR	NA	NA
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Lymphoma	PD	0	0.0
Acute Leukemia	PR	85	12.1
Acute Leukemia	CR	924	132
Acute Leukemia	PR	20	2.9
Acute Leukemia	PD	0	0.0
Acute Leukemia	PR	31	4.4
Acute Leukemia	CR	199	28.4
Acute Leukemia	CR	195	27.9
Acute Leukemia	CR	82	11.7
Acute Leukemia	PR	55	7.9
Acute Leukemia	PD	0	0.0
Acute Leukemia	CR	836+	119
Acute Leukemia	PD	0	0.0
Acute Leukemia	PR	237	33.9
Acute Leukemia	PR	39	5.6
Acute Leukemia	PR	84	12.0
Chronic Leukemia	PD	0	0.0
Chronic Leukemia	CR	233	33.3
Chronic Leukemia	PR	119	17.0
	Summaries		
	Mean	**49**	**6.9**
	Median	**10**	**1.4**
	Range	**0-924**	**0-132**

Table 4: Adverse Events in alemtuzumab in patients with ATL, grade 3, 4 or 5 AEs possibly, probably or definitely related to alemtuzumab therapy (N = 29)

Adverse Event	Grade 3 (%)	Grade 4 (%)
Hematologic:		
• Leukopenia	• 12 (41)	• 5 (17)
• Lymphocytopenia	• 17 (59)	• 0
• Neutropenia	• 9 (31)	• 1 (3)
• Anemia	• 7 (24)	• 0
• Thrombocytopenia	• 3 (10)	• 0
Hepatic:		
• Alkaline phosphatase	• 1 (3)	• 0
• Gamma-glutamyl transpeptidase	• 1 (3)	• 0
• Bilirubin	• 1 (3)	• 0
• Hypoalbuminemia	• 2 (7)	• 0
Metabolic:		
• Creatinine phosphokinase	• 1 (3)	• 0
• Hypercalcemia	• 1 (3)	• 0
• Hypocalcemia	• 1 (3)	• 0
• Hypokalemia	• 1 (3)	• 0
• Hypophosphatemia	• 1 (3)	• 0
Allergy Immunology:		
• Allergic reaction	• 1 (3)	• 0
Cardiovascular:		
• Vasovagal episode	• 2 (7)	• 0
• Hypotension	• 3 (10)	• 0
Constitutional symptoms:		
• Fever in the absence of neutropenia	• 3 (10)	• 0
Pulmonary:		
• Pulmonary infiltrates	• 1 (3)	• 0
• Pulmonary hypoxia	• 2 (7)	• 0
Ocular/Visual:		
• Blurred vision	• 1 (3)	• 0
• Photophobia	• 1 (3)	• 0
• (other) Uveitis	• 1 (3	• 0
Endocrine:		
• Hyperthyroidism	• 1 (3)	• 0
Dermatological:		
• Rash, desquamation	• 1 (3)	• 0
Infection:		
• With Grade 3 or 4 neutropenia	• 2 (7)	• 0
• Without Grade 3 or 4 neutropenia	• 2 (7)	• 0

16.8 APPENDIX H: SPECIAL FACS AND ASSAY FOR ADCC

- Peripheral blood mononuclear cells (PBMCs) should be isolated by Ficoll-High-Paque Density Gradient Centrifugation.
- The viable cells should be viably frozen and stored in liquid nitrogen.
- The ADCC assay will be performed on the same occasion for all samples of a given patient.
- Vials of frozen cells will be thawed using standard procedures 18 hours before the assay in accord with our experience with normal donors.
- 1.5 million of patient's PBMCs obtained before and on day 15 following IL-15 injection will be tested in aliquots as follows:
    - Tested alone
    - Tested with untreated Raji cells and with Raji cells coated with an antibody to CD20 for 5 hours.
    - In addition, we may utilize an ATL cell line in addition to Raji cells. These cell populations will be stained with CD107, CD3, CD56, and CD94.

16.9 APPENDIX I: MODIFIED SEVERITY WEIGHTED ASSESSMENT TOOL (MSWAT)

Body Region	% BSA in Body Region	Assessment of Involvement in Patient's Skin		
		Patch [a]	Plaque [b]	Tumor [c]
Head	7			
Neck	2			
Anterior trunk	13			
Arms	8			
Forearms	6			
Hands	5			
Posterior trunk	13			
Buttocks	5			
Thighs	19			
Legs	14			
Feet	7			
Groin	1			
Subtotal of lesion BSA				
Weighting factor		× 1	× 2	× 4
Subtotal lesion BSA × weighting factor				

Abbreviations: BSA = body surface area; mSWAT = Modified Severity Weighted Assessment Tool.

Note: mSWAT score equals summation of each column line.

a Any size lesion without induration or significant elevation above the surrounding uninvolved skin; poikiloderma may be present.

b Any size lesion that is elevated or indurated; crusting, ulceration, or poikiloderma may be present.

c Any solid or nodular lesion ≥1 cm in diameter with evidence of deep infiltration in the skin and/or vertical growth.

Source: Olsen et al. JCO. 2001;29(18):2598-2607