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Title: PHASE II TRIAL OF LMB-2, FLUDARABINE AND CYCLOPHOSPHAMIDE FOR ADULT T-CELL LEUKEMIA

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Drug Name:	LMB-2	Fludarabine (Fludara)	Cyclophosphamide (Cytoxan)
IND Number:	6662 - *Withdrawn 07/25/2017*		
Sponsor:	CTEP		
Manufacturer:	NCI	Generic	Generic
Supplier	NCI	NIH CC pharmacy	NIH CC pharmacy

Commercial Agents: None

PRÉCIS

Background:

- CD25 (p55, Tac or IL2Rα) is strongly expressed in virtually 100% of patients with adult T-cell leukemia/lymphoma (ATL), a highly aggressive HTLV-1 related malignancy responding poorly to chemotherapy.
- In ATL, the humanized anti-CD25 monoclonal antibody (Mab) daclizumab produced 13-14% responses, and the anti-CD52 Mab Alemtuzumab (Campath-1H) produced response lasting > 8 weeks in of 30% of 23 patients.
- LMB-2 is an anti-CD25 recombinant immunotoxin containing variable domains of murine MAb anti-Tac and truncated Pseudomonas exotoxin.
- In a phase I trial at NCI, the MTD of LMB-2 was 40 mcg/Kg·dose IV given every other day for 3 doses (QOD x3). LMB-2 induced > 90% tumor reduction rapidly in all 3 ATL patients on protocol but achieved only 1 partial response due to rapid tumor progression and/or immunogenicity.
- In preclinical models, response from recombinant immunotoxins is limited by high concentrations of soluble receptor in the blood and especially in the interstitial space of the tumor. Synergism was observed with chemotherapy and immunotoxins, possibly due reduction of soluble receptor in tumor interstitium.

Objective:

- To determine, in nonrandomized fashion, if after verifying its safety, fludarabine and cyclophosphamide (FC) prior to LMB2 for ATL can result in low immunogenicity and a rate of major response lasting > 8 weeks which may be an improvement over that demonstrated previously from CAMPATH.

Eligibility:

- CD25+ ATL, untreated or with prior therapy, leukemic type without malignant masses > 4 cm.
- ECOG 0-2, ANC, platelets and albumin at least 1000, 75,000, and 3.0, respectively.

Design:

- IV fludarabine and cyclophosphamide (FC) days 1 – 3 (doses listed respectively)
  - Patients 1-7 and 10 -14, and ≥18: 25 and 250 mg/m^2/day
  - Patients 8 – 9: 30 and 300 mg/m^2/day
  - Patients 15– 17: 20 and 200 mg/m^2/day
- LMB-2 dose: Begin with 30 mcg/Kg IV on days 3, 5 and 7. Escalate to 40 mcg/Kg if DLT in 0/3 or 1/6 at 30 mcg/Kg. Continue at 40 mcg/Kg if 0-1 of 6 have DLT at 40 mcg/Kg.
- Administer cycle 1 with FC alone. Two weeks after starting cycle 1, begin up to 6 cycles of FC plus LMB-2 at minimum 20-day intervals.
- Accrual goals: 29-37 evaluable patients, which includes 4 replacements.

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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

To determine, in nonrandomized fashion, if after verifying its safety, fludarabine and cyclophosphamide (FC) prior to LMB2 for ATL can result in low immunogenicity and a rate of major response lasting > 8 weeks which may be an improvement over that demonstrated previously from CAMPATH.

1.1.2 Secondary Objectives

- To determine the effect of 1 cycle of FC alone in ATL.
- To examine progression-free and overall survival in ATL after FC/LMB-2.
- Evaluate pharmacokinetics, toxicity, and monitor soluble CD25 and other tumor marker levels in the serum.
- To study the effects of LMB-2+FC on normal B- and T-cell subsets by FACS.

1.2 BACKGROUND

1.2.1 Introduction to CD25

The interleukin-2 receptor (IL2R), which binds IL2 with high affinity, is composed of a complex of alpha (CD25), beta (CD122) and gamma (CD132) subunits [1]. IL2 binds to CD25 with low (Kd ~ 10^{-8} M), to the complex of CD122 and CD132 with intermediate affinity (Kd ~ 10^{-9} M), and to the complex of CD25, CD122, and CD132 with high affinity (Kd ~ 10^{-11}). However, the anti-CD25 Mab anti-Tac [2-6] binds CD25 alone with high affinity (Kd ~10^{-10} M).

1.2.2 Introduction to ATL

ATL is an aggressive CD4+/CD25+ T-cell lymphoproliferative disorder caused by human T-cell lymphotropic virus type I (HTLV-I) [7, 8]. Cases are clustered in southwestern Japan, the Caribbean basin, northeastern South America, central Africa, and the

southeastern United States [9]. An individual infected with HTLV-1 appears to have an approximately 0.1 percent per year risk of developing frank ATL, a cumulative lifetime risk of 2 to 5 percent.

1.2.3 Mechanism of CD25 positivity in ATL

The HTLV-I genome contains pX, which encodes nonvirion proteins including 42-kDa Tax and 27-kDa rex. Tax is a trans-activating transcription factor that activates the HTLV-I long terminal repeat (LTR), the expression of viral genes, and the transcription of cellular genes including those encoding IL-2Rα (CD25), IL-2, IL-3, IL-15, IL-15Rα, TNF, GM-CSF, TGF-1, PTHrP, vimentin, and c-fos [10]. HTLV-I infection of T cells in vivo and in vitro leads to constitutive IL-2Rα gene expression and facilitates IL-2 expression, thereby leading to T-lymphocyte immortalization. It has been proposed that in the early phases of ATL the HTLV-I-induced leukemogenesis may be the result of Tax expression through its stimulatory effects on genes involved in cellular proliferation. Specifically, Tax expression may begin a process of cellular proliferation via the membrane-localized IL-2/IL-2R interaction with subsequent events required for frank malignancy.

1.2.4 Clinical Features of ATL

Acute ATL usually occurs 20-30 years following perinatal infection from the mother infected with HTLV-I. Clinical features include skin involvement, moderate lymphadenopathy with relative sparing of the mediastinum, CNS and lung involvement, hepatosplenomegaly, and hypercalcemia [7, 8]. Circulating ATL counts may be high without much anemia and with only modest involvement of the bone marrow. Profound immunosuppression results in opportunistic infections including Pneumocystis carinii and Cryptococcus meningitis [11]. Nevertheless, ATL patients are still capable of humeral immunity, as evidenced by HAMA responses to murine anti-Tac [12-14] and anti-toxin response to LMB-2 [15]. Four categories of aggressiveness include: 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 x 109/L), lack of hypercalcemia, lactic dehydrogenase (LDH) values no greater than 1.5 times the normal upper limit (ULN), 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. 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 ULN, and no hypercalcemia or involvement of the central nervous system, bone or gastrointestinal tract or manifestation 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.

1.2.4.1 Chemotherapy treatment of ATL

As reviewed recently [16], there is no effective treatment for ATL which significantly improves prognosis. A common first-line therapy is CHOP, and CHOP alone produced a CR rate of 40%, median survival was only 8 months and PFS was not reported [17]. Intensification of CHOP with VP16, vindesine, ranimustine and mitoxantrone increased CR rates but median survival remained low at 8-8.5 months. A 13 month median survival was achieved with a highly intense regimen of VCAP (vincristine, cyclophosphamide,

doxorubicin, and prednisone), AMP (doxorubicin, ranimustine, and prednisone), and VECP (vindesine, etoposide, carboplatin, and prednisone), but with grade 4 hematologic toxicity in most patients. The purine analog pentostatin (2 deoxycoformycin, DCF) which inhibits adenosine deaminase induced 3/18 PRs in a phase I trial, and 2/25 CR + 1/25 PR in another trial. Pentostatin combined with chemotherapy (vincristine, doxorubicin, etoposide, and prednisolone) resulted in 52% of 60 patients achieving CR, but the median survival was only 7.4 months [18]. The topoisomerase I inhibitor CPT-11 induced 1/13 CR and 4/13 PR in one study. Treatment with zidovudine and interferon achieved CR/PR rates up to 60% and possibly higher when preceded by CHOP, but median survival remained < 1 year. A zidovudine-interferon trial performed at NIH in 18 previously treated ATL patients showed 1 CR and 2 PRs, with median PFS < 1 month [19]. The most aggressive large trial of chemotherapy in ATL was a phase III trial comparing alternating vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP), doxorubicin, ranimustine, and prednisone (AMP), and vindesine, etoposide, carboplatin and prednisone (VECP) with biweekly CHOP [20]. This trial showed that the CR+CRu rate was higher in the VCAP-AMP-VECP group (40%) than the CHOP group (25%, p = 0.02), with 3-year overall survival (OS) rates of 24 vs 13% (p = 0.086). The probability of 1 year PFS was 28% and 16%, and 65% of the patients were progression free at 4.6 and 4.1 months, respectively. However, all patients were previously untreated and toxicity was very high with 98-83% grade 4 neutropenia, 32-17% grade 4 thrombocytopenia, and 32-15% grade 3-4 infection, respectively, and 3 toxic deaths reported in the VCAP-AMP-VECP arm. Finally allotransplant is used for ATL with 3-year DFS of 34%, but the median PFS was only 9 months and 65% of the patients had a PFS of only 4.6 months.

1.2.5 Monoclonal antibody treatment of ATL

The murine anti-Tac (anti-CD25) Mab resulted in 2 CRs and 4 PRs in 19 patients [12, 13]. ^{90}Y-murine anti-Tac resulted in 2 CR and 7 PRs in 18 patients [14]. In this trial, the median PFS was 5.5 months but at 1 month only 65% of had PFS. Humanized anti-Tac (Zenapax, daclizumab) was tested in a phase I trial of 14 ATL patients, with 2 PRs, and in a phase II trial 0 of 9 acute/lymphoma and 2 of 7 chronic/smoldering ATL patients responded. The anti-CD52 Mab Alemtuzumab (Campath-1H) has been tested in 24 ATL patients, mostly previously treated, on an NIH Phase II study (NCI 03-c-0194). Of 23 evaluable, 10 (46%) responded but only 7 (30%) for > 2 months. The median PFS in the first 22 patients was 1.8 months. Moreover 65% of these patients had a PFS of < 1 month. NCI 03-c-0194 has a maximum accrual of 30 patients.

1.2.6 Targeting CD25 in disorders other than ATL

The naked murine Mab anti-Tac and its humanized form (daclizumab, Zenapax) were tested in patients with renal transplantation [21-23], and the latter was approved by the FDA in 1997 for prevention of renal allograft rejection [24]. ^{90}Y-anti-Tac produced 29% PRs and 41% CRs out of 17 patients with Hodgkin's disease (HD) [24]. IL2 fused to either the first 485 or 388 amino acids of diphtheria toxin showed clinical activity in patients with CD25+ hematologic malignancies [25-30] and the latter agent, $DAB_{389}IL2$ (Ontak, denileukin diftitox) was approved for use in patients with CTCL. Denileukin diftitox produced 10% CRs and 20% PRs in CTCL [29, 30], 17% PRs in CLL [31], and 7% CRs and 18% PRs in NHL [32]. Finally, the anti-CD25 Mab RFT5 conjugated to deglycosylated ricin A chain (RFT5-dgA) induced 11% PRs in patients with HD [33-36].

The dose-limiting toxicity of RFT5-dgA was vascular leak syndrome (VLS). Denileukin diftitox caused less frequent and less severe VLS, with most patients (92%) having constitutional and gastrointestinal symptoms consisting of fever, chills, asthenia, nausea, vomiting, arthralgia, headache, diarrhea, and anorexia [29].

1.2.7 Development of Pseudomonas exotoxin (PE) for targeting cells

The full-length 613 amino acid PE protein contains three functional domains which are necessary for cellular intoxication [37, 38]. Domain Ia (amino acids 1-252) is the binding domain, domain II (amino acids 253-364) is for translocating the toxin to the cytosol and domain III (amino acids 400-613) contains the ADP ribosylating enzyme which inactivates elongation factor 2 (EF-2) in the cytosol and results in cell death. The function of domain Ib (amino acids 365-399) is unknown. A current model of how PE kills cells includes the following steps: 1) The C-terminal residue (lysine-613) is removed by a carboxypeptidase in the plasma or culture medium [39]. 2) Domain Ia binds to the a2 macroglobulin receptor, present on animal cells [40]. 3) After internalization at low pH, domain II is proteolytically cleaved between amino acids 279 and 280 by furin [41-43]. 4) The disulfide bond between cysteines 265 and 287 which joins the two fragments is reduced, producing an N-terminal fragment of 28 kDa and a C-terminal fragment of 37 kDa. 5) Amino acids 609-612 (REDL) bind to an intracellular sorting receptor which transports the 37 kDa carboxy terminal fragment from the transreticular Golgi apparatus to the endoplasmic reticulum [44, 45]. 6) Amino acids 280-313 mediate translocation of the toxin to the cytosol [46, 47]. 7) The ADP ribosylating enzyme composed of amino acids 400-602 inactivates EF-2 [48]. PE40 is a truncated derivative of PE which is missing the binding domain and hence will not bind specifically to cells unless attached to an antibody or growth factor [37, 49]. PE38 is a truncated version of PE40 which is missing amino acids 365-380. While PE40 and PE38 have similar activities [50, 51], we have preferred to use PE38 because it is slightly smaller and is missing a disulfide bond that impairs refolding the protein.

1.2.8 Preclinical development of LMB-2

To target IL2R+ disorders expressing CD25 regardless of the presence of other subunits of the IL2R, the anti-CD25 MAb anti-Tac was used as a ligand instead of IL2. The rationale is based on the higher binding of CD25 alone to anti-Tac (Kd ~ 10^{-10} M) than to IL2 (Kd = 10^{-8} M) [4]. CD25 greatly outnumbers CD122 and CD132 on most malignant cell types [52, 53]. Although early studies indicated CD25 alone would not internalize anti-Tac [54], CD25 alone does internalize bound recombinant toxin [51, 55, 56]. A recombinant single-chain Fv [57, 58] was constructed containing the variable heavy domain (V_H) fused to the variable light domain (V_L) via the peptide linker $(G_4S)_3$, and V_L was fused to truncated PE [59]. The resulting recombinant immunotoxin anti-Tac(Fv)-PE40 and its slightly shorter derivative anti-Tac(Fv)-PE38 (called LMB-2) were selectively cytotoxic toward CD25+ malignant cell lines and toward leukemic cells freshly obtained from patients [51, 56, 60-63]. Antitumor studies in mice bearing CD25+ xenografts showed complete regressions, and biodistribution studies showed concentration of LMB-2 into such tumors *in vivo* [56, 61]. Primary adult T cell leukemia (ATL) and hairy cell leukemia (HCL) cells were much more sensitive than primary CLL cells, probably due to lower CD25 expression in the latter. Administration of LMB-2 to Cynomolgus monkeys at 300 mcg/Kg every

other day (QOD) x 3 doses caused transaminase elevations and loss of appetite, but was not lethal even at doses of 750 and 1000 mcg/Kg QOD x3 days [62].

1.2.9 Phase I trial of LMB-2 (NIH #96-C-0064): response and immunogenicity

LMB-2 induced responses in patients with CD25+ chemotherapy-resistant hematologic malignancies, including 4 with hairy cell leukemia (1 CR, 3 PR) and one PR each with adult T-cell leukemia, Hodgkin's disease, chronic lymphocytic leukemia, and cutaneous T-cell lymphoma [15]. The published results included 35 patients (age range 24-79), 11 with HD, 6 with B-cell lymphoma, 8 with CLL, 4 with HCL, 3 with PTCL, 1 with CTCL, and 2 with ATL. Of these 35 patients, 22 received 1 cycle only, 8 received 2 cycles, 2 received 3 cycles, and 1 each received 4, 5, and 6 cycles. Four additional patients were treated after publication of the phase I trial, including 1 each with ATL, HD, CTCL and NHL. Of the 39 patients, after a single cycle, 9 (23%) had low and 6 (15%) had high levels of neutralizing antibodies, and after all treatment, 8 (21%) had low and 12 (31%) had high levels of neutralizing antibodies. Low and high levels of neutralizing antibodies were defined as > 50% neutralization of 200 ng/ml and > 75% neutralization of 1000 ng/ml of the cytotoxicity of LMB-2 toward CD25+ cultured cells. Thirty-four patients were tested by ELISA assay after all treatment for antibodies binding to the toxin, and for antibodies binding to murine IgG epitopes (HAMA) [15]. Low and high levels of binding antibodies were defined by titers of from 1:10,000 to 1:400,000 and > 1:400,000, respectively. Out of 34 patients, 10 (29%) had low and 10 (29%) had high titers of binding antibodies to toxin (PE38), and 4 (12%) had low and 5 (15%) had high titers of HAMA [15]. No patient had HAMA without binding antibodies to the toxin or without neutralizing antibodies, but 4 patients with low titers of binding antibodies to the toxin had no neutralizing antibodies or HAMA.

1.2.10 LMB-2 related toxicity

All toxicity was reversible. The maximum tolerated dose (MTD) was 40 mcg/Kg QOD x3, where the most common toxicities were transaminase elevations (92%), fever (83%), myalgias (58%), hypoalbuminemia (58%), nausea (42%), and weight gain (42%). Transaminase elevations were generally asymptomatic and always reversible. Hypoalbuminemia and weight gain were evidence of a form of VLS much milder than that described for the larger toxin chemical conjugates containing whole Mab [36, 64]. DLT was observed in two of three patients at the 63 mcg/Kg·dose QOD x 3 dose level. The first patient (#31, with HD) had asymptomatic grade 4 AST and grade 3 ALT elevations. The second patient (#32, with HCL) developed grade 3 diarrhea, grade 2 fever, nausea, and vomiting, and grade 4 cardiomyopathy on day 5. The patient's cardiac function returned to normal by day 7. FACS of peripheral blood on day 9 indicated a large number of dead tumor cells. We concluded that the cardiomyopathy was probably cytokine mediated rather than direct LMB-2 toxicity on the heart. The 50 mcg/Kg dose level was dose limiting in 1 of 6 patients (patient #27 with PTCL) due to an allergic reaction.

1.2.11 Pharmacokinetics of LMB-2

For pharmacokinetic studies, dilutions of patient plasma were tested in cytotoxicity assays against a cell line (SP2/Tac) and compared to a cytotoxicity standard curve created with known amounts of LMB-2. In 12 patients treated at the MTD (40 mcg/kg), peak plasma levels after cycle 1 were 340-1040 (median 560) ng/ml, the AUCs were 48-257 (median

123) ug-min/ml, the half-lives were 185-322 (median 216) min, and the clearances were 14-79 (median 25) ml/min). The median values for the 4 patients with HD were 620 ng/ml, AUC 155 ug-min/ml, half-life 193 min, and clearance 19 ml/min.

1.2.12 Additional patients #36-39 on the phase I trial

To determine if LMB-2 would be better tolerated if patients received prophylactic i.v. fluid, an additional 4 patients were enrolled. Patient #36 with ATL was treated at 50 mcg/Kg QOD x3 and experienced a reversible but dose-limiting syndrome similar to patient #32, with 3rd spacing and muscle edema causing grade IV CK elevation and hypoventilation leading to supraventricular tachycardia and respiratory failure. This patient had a muscle biopsy which ruled out necrosis or inflammation, indicating that the CK elevation was only from passive muscle fiber edema and stretching. Patients #37-39 with HD, CTCL and NHL received 40 mcg/Kg·dose QOD x3 with prophylactic fluid, and tolerated LMB-2 well.

1.2.13 Phase II testing of LMB-2 in CLL and CTCL

LMB-2 has been administered to 13 CLL patients without DLT at 40 mcg/Kg QOD. One patient had 6 cycles and a PR. Four additional patients had > 50% reductions in circulating CLL cells but did not meet criteria for PR due to limited response duration or due to lymph nodes which did not regress by > 50%. However, 2 additional patients did achieve significant reductions in lymph nodes on LMB-2. On a phase II HCL protocol for patients with prior BL22, LMB-2 produced PD in one patient and PR in another. On a phase II CTCL protocol, 1 patient was enrolled at 40 mcg/kg QOD x3 who had transient grade IV CPK elevation as his only DLT, so the dose level was lowered for an additional 4 CTCL patients to 30 mcg/Kg QOD x3. None of these patients experienced DLT. All 5 patients experienced a decrease in itching or regression of skin lesions within 1-2 weeks of their first dose of LMB-2. However, 4 of 5 patients developed neutralizing antibodies after the first 1-2 cycles of LMB-2, thus preventing cumulative major responses. The higher rate of neutralizing antibodies in CTCL than CLL is attributed to the likely exposure of patients to *Pseudomonas* aeruginosa via skin infections, and the consequent development of low levels of neutralizing antibodies prior to treatment with LMB-2.

1.2.14 Pilot trial of LMB-2 after FC in HD

To determine if immune suppression with FC would prevent immunogenicity from LMB-2, a pilot trial in HD was proposed and approved in which patients would receive FC at 30 and 600 mg/m^2 qd x4 at least 27 days prior to LMB-2. However, after screening > 10 patients, it was found that the true rate of CD25 positivity on the malignant cells was very low (< 20%), and that most CD25+ HD patients had CD25 only on the surrounding T-cells. Also since this trial was approved, preclinical data emerged suggesting that high concentration of soluble receptor in the interstitial space of the tumor is a major limiting step in immunotoxin efficacy [65]. These data suggested that to decrease immunogenicity and increase response, chemotherapy should be given with each cycle of LMB-2 rather than prior to all cycles. Because of the difficulty in performing a trial in a single disease (HD) which has such a low rate of eligibility, the HD trial was closed prior to accruing any patients.

1.2.15 Treatment of lymphoma and CLL with FC

Although trials of FC in ATL have not been reported, the following trials report safety and efficacy of FC in other hematologic malignancies:

1.2.15.1 In 1999 several small trials showed response rates of 50-74% with FC in patients with indolent and aggressive lymphomas and CLL [66, 67].

1.2.15.2 In 60 untreated patients with indolent lymphoma and CLL, FC at 20 mg/m^2 d1-5 and 600 mg/m^2 d1 resulted in 51% CRs and 41% PRs. GCSF 5 mcg/Kg/day was started on day 8 [68]. The mean CD4 count dropped from 799 to 139/ul, IgG from 1110 to 907 mg/dL, and IgA from 129 to 99 mg/dl.

1.2.15.3 A 100% response rate to FC at 20 mg/m2 d1-5 and 600-1000 mg/m2 day 1 was reported in indolent lymphoma patients without prior chemotherapy, including 89% CRs and 11% PRs [69].

1.2.15.4 In 30 patients with MCL, half of whom had >1 prior therapy, there were 30% CRs and 33% PRs, and the CR rate was 100% in the 10 without prior chemotherapy [70]. These patients were treated with 3-4 week intervals of fludarabine 20-25 mg/m^2 qd x3-5 and cyclophosphamide either 275 mg/m^2 qd x3 or 500-1000 mg/m^2 x1. Although FC is usually administered every 4 weeks, in this trial and in others for MCL where fludarabine is combined with idarubicin [71], the interval of 3 was well tolerated.

1.2.15.5 In 22 patients with recurrent indolent NHL treated with FC at 25 mg/m^2 d1-3 and 300 mg/m^2 d1-3, CR and PR rates of 60 and 30% were reported [72].

1.2.15.6 In 128 patients with CLL treated with FC at 30 and 500-300 mg/m^2 on d1-3 q4-6w, the ORR was ≥ 80% and 38% in patients who were responsive or refractory to prior fludarabine [73]. The cyclophosphamide dose had to be reduced from 500-300 mg/m^2 due to cumulative myelosuppression.

1.2.15.7 In 27 patients with indolent NHL treated with FC at 30 and 250 mg/m^2 on d1-3 q4w, half of whom were previously untreated, 41% CRs and 13% PRs were observed, and the ORR was 93 vs 85% in patients without or with prior therapy [74]. In this trial, the median CD4 and CD8 count decreased from 750 and 856 to 155 and 204/ul.

1.2.15.8 At a slightly lower dose level, 64 patients with CLL and indolent NHL were treated with FC at 25 and 250 mg/m^2 d1-3 q4w, with CR and PR rates 86 and 29% [75].

1.2.15.9 To determine the effect of GM-CSF to prevent infections, 34 patients with previously treated indolent NHL and CLL received FC at 30 and 300 mg/m^2 d1-3 q4w and were randomized to receive GM-CSF at 250 mcg/m^2/day from day 4 to 2 days before the next cycle. CR and PR rates were 26 and 52% and GM-CSF did not lower the incidence neutropenic infections [76].

1.2.15.10 In 32 elderly CLL patients treated with FC at 25 mg/m^2 d1-5 and 250/m^2 d1-3 q4w, CR and PR rates were 0 and 59%, and a high incidence of infections were observed [77].

1.2.15.11 In 11 patients with WM treated with FC at 25 mg/m^2 and 250 mg/m^2 d1-3 q4w x4 cycles, a PR rate of 55% was observed and FC was found to be well tolerated [78].

1.2.15.12 Treatment of indolent NHL with oral FC. To determine the benefit of oral FC, 12 patients received oral FC at 40 and 300 mg/m^2 d1-3 q4w x 6 cycles, and CR and PR rates were 66 and 0% [79]. In that trial, the CR rate was 83% for 6 with only 1 prior therapy, versus 50% for 6 with 2 or more prior therapies. Toxicity was mild, including neutropenia in 25% and grade 1-2 thrombocytopenia in only 8%. Nausea and vomiting were

controlled. In a larger study, 25 elderly patients with untreated indolent NHL were treated with oral FC at 25 and 150 mg/m^2 d1-4 q4w, resulting in 40% CRs and 44% PRs [80]. The bioavailability of oral Fludarabine and Cyclophosphamide is approximately 55% [81] and 85% [82].

1.2.15.13 Treatment of T-cell malignancies with FC. Patient with T-cell lymphomas are responsive to Fludarabine but there are few trials of FC in such patients. In a study of 12 patients with CTCL treated with FC at 18 and 250 mg/m^2 d1-3 q4w, 5 of 6 who tolerated 3 cycles responded, with 1 CR and 4 PRs [83]. In a case report, a patient with PTCL resistant to CHOP and ESHAP had a 14-month CR to FC at 20 mg/m^2 d1-5 and 600 mg/m^2 d1, given for just 1 cycle [84].

1.2.15.14 Doses of FC used in FCR trials. More recently, FCR has been used for B-cell lymphomas and CLL, and the most common dose of FC was 25 + 250/m^2 qd x3. 177 pretreated patients with CLL were treated with FCR at 25 mg/m^2 day 2-4, 250 mg/m^2 d2-4, and 375 mg/m^2 d1 of cycle 1 followed every 4 weeks by cycles 2-6 of the same doses given d1-3, d1-3 and d1 [85]. 224 patients with previously untreated CLL were treated with FCR at 25-30 mg/m^2 day 2-4, 250-300 mg/m^2 d2-4, and 375 mg/m^2 d1 of cycle 1 followed every 4 weeks by cycles 2-6 of the same doses given d1-3, d1-3 and d1 [86]. 76 patients with CLL, FL and other lymphoid malignancies received a median of 4 cycles of FCR at 25 mg/m2 day 1-3, 250 mg/m2 day 1-3, and 375 mg/m2 day 1 [87].

1.2.16 Immunogenicity of Mabs and immunotoxins

1.2.16.1 Human anti-murine antibodies (HAMA). Although now essentially circumvented by humanized and murine-human chimeric antibodies, HAMA was a major issue preventing effective use of murine Mabs for cancer detection and treatment. For example 100% of 24 patients with colon cancer treated with the radiolabeled murine anti-TAG-72 Mab I-131-CC49 had HAMA within 4 weeks of treatment [88]. Using the murine Mab T101 Mab, 100% of 4 CTCL and 0% of 4 CLL patients made HAMA, indicating that the hypogammaglobulinemia associated with CLL prevented HAMA [89].

1.2.16.2 Immunogenicity of immunotoxins. Conventional immunotoxins composed of whole murine Mabs connected to toxins, like anti-B4-Blocked ricin, exhibited humoral immunity against the antibody or toxin, or both . [90]. High rates (75%) of antibodies were observed with immunotoxins in metastatic colon cancer [91] and lower rates were observed in B-cell lymphoma [92]. In the conventional immunotoxin LMB-1, containing whole anti-Le^Y murine Mab connected to truncated Pseudomonas exotoxin, one cycle of therapy led to high levels of neutralizing antibodies in 91% of patients [93]. HAMA could be reduced or eliminated by using recombinant immunotoxins, which contain only the Fv domain of the Mab. However, the toxin is still immunogenic. The recombinant immunotoxin SS1P, containing the anti-mesothelin Fv fused to truncated Pseudomonas exotoxin, induced neutralizing antibodies in 88% of patients after 1 cycle [94].

1.2.16.3 Immunogenicity of LMB-2. As mentioned in section **1.2.9**, of the 39 patients treated on the phase I trial of LMB-2, after a single cycle, 9 (23%) had low and 6 (15%) had high levels of neutralizing antibodies, and after all treatment, 8 (21%) had low and 12 (31%) had high levels of neutralizing antibodies. Low and high levels of neutralizing antibodies were defined as > 50% neutralization of 200 ng/ml and > 75% neutralization of 1000 ng/ml of the cytotoxicity of LMB-2 toward CD25+ cultured cells. Thirty-four patients were tested by ELISA assay after all treatment for antibodies binding to PE38, and for antibodies binding to murine IgG epitopes (HAMA) [15]. Low and high levels of binding antibodies were defined by titers of from 1:10,000 to 1:400,000 and > 1:400,000, respectively. Out of 34 patients, 10 (29%) had low and 10 (29%) had high titers of binding antibodies to PE38, and 4 (12%) had low and 5 (15%) had high titers of HAMA [15]. No patient had HAMA without binding antibodies to PE38 or without neutralizing antibodies, but 4 patients with low titers of binding antibodies to PE38 had no neutralizing antibodies or HAMA. On the phase II CTCL trial, 4 (80%) of 5 patients made high levels of neutralizing antibodies after 1 cycle. On the phase II CLL trial, only 2 (15%) of 13 patients made high levels of neutralizing antibodies, each after 6 cycles. On the phase II HCL trial, 1 (50%) of 2 patients have made low levels of neutralizing antibodies after 2 cycles.

1.2.17 Reducing humoral immunity with chemotherapy and other agents

1.2.17.1 Cyclosporine A. Patients receiving a radiolabeled anti-CEA Mab received cyclosporine A to prevent HAMA. Although HAMA was not eliminated, it was lessened in severity so that more Mab could be given [95]. In another study, cyclosporine given to 13 patients treated with murine antibody fragments was associated with HAMA in 62%, vs 86% in patients not receiving cyclosporine [96]. However, the cyclosporine was complicated by hyperbilirubinemia, renal insufficiency, and hypertension.

1.2.17.2 15-deoxyspergualin is an agent which was shown to prevent HAMA in preclinical models, and was found in mouse studies to prevent neutralizing antibodies to PE and LMB-1 [97]. However, later experiments in monkeys showed no prevention of immunogenicity.

1.2.17.3 Cyclophosphamide. It was recently reported that in patients receiving the anti-neuroblastoma murine Mab 3F8 after chemotherapy, HAMA incidence was lower if patients received high dose cyclophosphamide prior to 3F8 ($p < 0.001$) [98]. It was found that HAMA was least likely if 3F8 followed cyclophosphamide by < 90 days. In this study, high dose cyclophosphamide was given at > 4000 mg/m^2 or > 140 mg/Kg per cycle.

1.2.17.4 Fludarabine. In a trial of the anti-CD20 murine radiolabeled Mab Tositumomab following fludarabine for untreated FL, it was found that only 2 (6%) of 35 patients developed HAMA [99]. By comparison, 65% of untreated FL patients got HAMA from Tositumomab when fludarabine was not used. The dose of fludarabine used in this study was 25 mg/m^2 qd x5, and was given only once, 5 weeks before the tositumomab.

1.2.17.5 Rituximab. The anti-CD20 human-murine chimeric Mab Rituximab effectively depletes normal B-cells [100], raising the possibility that it could be used to blunt humoral immunity from immunotoxins. A prospective trial was done to determine if Rituximab could block the immunogenicity of LMB-1, and it was found that it could not [101]. However, it is possible that Ritixumab could decrease the risk of immunogenicity of LMB-2, which is smaller and less immunogenic than LMB-1.

1.2.17.6 Treatment with higher-dose FC specifically to decrease immunity. Breast cancer patients have been treated with 1-2 cycles of fludarabine 30 mg/m^2 and cyclophosphamide 600 mg/m^2, each qd x4 as part of induction therapy for stem cell transplantation (SCT) [102, 103]. By day 17 after induction, not only did the median CD4+ lymphocyte count drop from 452 to 41/ul, but the CD8+ lymphocyte count dropped from 259 to 47/ul, and B-cells dropped from 153 to only 1/ul. CD4 counts of < 50/ul were achieved in 13 of 17 breast cancer patients after 1 cycle.

1.3 RATIONALE

1.3.1 Choice of ATL as a disease model to test LMB2 + chemotherapy

This protocol tests a 2-pronged approach to improve the activity of LMB-2 toward CD25+ malignancies. First, the FC regimen is known to reduce both T- and B-cell numbers which may decrease immunogenicity. In fact, even patients with prior exposure and a low-level of antibodies to PE may not produce a secondary immune response after FC. Second, LMB-2 has difficulty reaching tightly packed tumor cells and may work better when the tumor cells are disrupted with chemotherapy, decreasing local tumor concentrations of sCD25. ATL is an excellent model to test this hypothesis, first because of extremely high and essentially universal sensitivity of ATL cells to LMB-2 and similar recombinant immunotoxins targeting CD25 [51, 63, 104-107]. Second, all of the 3 phase I ATL patients responded with rapid > 90% reductions in tumor burden during the first cycle of LMB-2, but major response was limited to only 1 PR due to rapid regrowth of tumor. Third, many patients with ATL have solid tumor masses, and high local concentrations of sCD25 could be a limiting factor preventing optimum response, and could be prevented by FC prior to LMB-2. This hypothesis is based on published animal data showing soluble mesothelin levels in tumor interstitium 10-fold higher than serum soluble mesothelin levels, and synergism between chemotherapy and anti-mesothelin recombinant immunotoxins SS1P [65]. Moreover, unpublished animal data show soluble CD22 levels 100-fold higher in tumors than in serum, as well as synergism between chemotherapy and BL22. Fourth, ATL patients are known to have the highest concentrations of sCD25 in the blood [108], which would prevent LMB-2 from reaching tumor cells, and tumor burden with sCD25 could be at least transiently reduced with FC prior to LMB-2. Because of its small size (45 kDa) and short half-life [108], rapid reductions in tumor burden should result in rapid clearing of sCD25. Fifth, 2 out of 3 phase I patients with ATL produced either low or high levels of neutralizing antibodies to 1-2 cycles of LMB-2, suggesting that preventing the immunogenicity of LMB-2 with FC could improve response. Finally, we have reported that the related anti-CD22 recombinant immunotoxin BL22 can induce CR in a high percentage of patients with chemoresistant hairy cell leukemia (HCL) [109, 110], and CD25 expression on ATL is similar to CD22 expression on HCL. LMB-2 could actually be more effective for ATL than BL22 is for HCL, if the problems of sCD25, rapid tumor progression, and immunogenicity could be eliminated by FC pretreatment. This is because while HCL cells in some patients could originate from CD22 negative clones, the clonogenic ATL cells are clearly CD25 positive. Also, ATL is a disease which lacks effective standard therapy with median survivals of only 4 months for acute and 9 months for lymphomatous subtypes. Innovative approaches in this disease are clearly needed.

1.3.2 Frequency of administration of LMB-2

An important goal of this trial is to administer multiple cycles of LMB-2, since phase I-II data indicate suboptimal response with 1 cycle, and testing prevention of immunogenicity requires repeated dosing. For many tumors which responded at 7 days but progressed by 28 days, retreatment intervals as short as 3 weeks would be better. This should be well tolerated given the rapid reversibility of LMB-2 toxicity. The LMB-2 phase I protocol was amended to deliver LMB-2 at 2 week intervals to patients with ATL, although the trial was completed before additional ATL patients were enrolled.

1.3.3 Rationale of choice of FC regimen

Based on the clinical efficacy of chemotherapy to prevent immunogenicity (section **1.2.17**), and the known ability of FC to reduce normal T- and B-cells, we believe the FC combination is best to use for prevention of immunogenicity and for decreasing tumor burden/sCD25 prior to LMB-2. There is extensive experience, reviewed above, in using FC for B-cell malignancies and the doses chosen, 25 + 250 mg/m^2 qd x3, are at the low end of those tested. Although FC is usually given at 4 week intervals, it has been observed at NIH that ATL rapidly progresses between cycles if spaced at 4 weeks, even if using dose-intense regimens like F-EPOCH. High dose FC (30 + 600 mg/m^2 qd x4) as used at NIH [102, 103] is excellent at decreasing T- and B-cells (see section **1.2.17.6**), and has been well tolerated in breast cancer patients given every 3 weeks for up to 3 cycles. We believe that this regimen would be too toxic in patients with prior chemotherapy for ATL, and it could not be administered 6 times. Even though FC should cause T-cell depression lasting much longer than a month [111], the potential antitumor benefit of FC would need to be renewed as frequently as possible. Therefore, we have selected FC at 25 + 250 mg/m2 for 6 cycles every 3 weeks. It was found by the NCI MOB transplant group that cyclophosphamide dosage was most critical for immune depletion. The FC combination of 30 + 350/m2 qd x3 was used in a trial of 128 patients with CLL pretreated with fludarabine (see **1.2.15.6**) [73], and although it was more toxic than 30 + 300/m^2, FC at 25 + 350 mg/m^2 may be well tolerated in ATL patients.

1.3.4 Combined therapy with LMB-2 and chemotherapy

Combined therapy with LMB-2 and chemotherapy could be synergistic in patients due to several mechanisms. First, high pressures inside the tumor could be liberated by chemotherapy. However, a much more relevant problem may be soluble receptor which can reach very high interstitial concentrations within the tumor and present a block to the movement of recombinant immunotoxin toward the tumor cell. This hypothesis is based on experiments analyzing soluble mesothelin in the extracellular space of tumors [65]. Unpublished experiments have already documented the same problem in the targeting of BL22 to CD22+ solid tumors in mice. The experiments indicate that synergism between chemotherapy and immunotoxins *in vivo* could be related to tumor disruption by chemotherapy, releasing the high concentration of extracellular antigen, and rendering the tumor cells more susceptible to immunotoxins.

1.3.5 Antitumor activity in LMB-2 patients

As shown in **Table 1.A, Table 1.B** and **Table 1.C**, a total of 34 patients had some evidence of antitumor activity by LMB-2 including 24 patients who failed to achieve a major response. These 34 patients include 21 (64%) of the 39 on the phase I trial, 7 (54%) of the

13 on the phase II CLL trial, all 5 on the CTCL trial, and 1 of 2 on the phase II HCL trial. These 3 phase II trials test LMB-2 alone and are still open. The CLL trial is nearing completion with only 4 of 17 slots remaining, the CTCL trial is accruing slowly due to competing trials, and the HCL trial is limited mainly to patients who have experienced HUS with BL22. The reasons for treatment failure in patients without major response might include problems with tumor penetration due to hydrostatic pressure and high interstitial sCD25. Immunogenicity is a limiting factor in all 3 trials, particularly the CTCL trial where it has limited retreatment in nearly all patients. Two patients, L216 and L219, each had PD after having transient tumor reduction, and also had low levels of neutralizing antibodies. Combined treatment with LMB-2 after FC may address both of these problems. In addition to these 34 patients who might have benefited by pretreatment with FC, one patient on the phase II HCL trial (HC01) had PD despite high sensitivity *ex vivo* of his HCL cells to LMB-2 (IC50 = 3.6 ng/ml). This patient had a sCD25 level of 235 ng/ml in the serum prior to LMB-2, which likely prevented response. Thus if FC can decrease both tumor burden, sCD25, and immunogenicity, it could have beneficially affected the majority (58%) of the 59 patients previously treated with LMB-2 on 4 protocols. Therefore, our hypothesis is that FC at 25 and 250 mg/m2 will decrease the immunogenicity of LMB-2 and prolong its antitumor activity based on in vivo experiments in a related system [65]. If so, current trials administering LMB-2 alone could be closed and chemotherapy could be made a component of LMB-2 treatment of diseases other than ATL in future trials.

1.3.6 Eligibility for Acute and Chronic ATL

As noted above in **1.2.4**, ATL has been described with 4 prognostic subtypes, and only the smoldering subtype, is somewhat favorable [17, 112, 113]. The chronic subtype, featuring malignant lymphocytosis over 3.5 x 10^9/L or LDH between 1.5 and 2.0-fold elevated but not hypercalcemia, still has a median survival of only 24 months for Japanese and 21 months for Caribbean patients with ATL. Thus, the chronic subtype of ATL, though less severe than the acute subtype, would still clearly be appropriate for testing with FC/LMB2. Patients with smoldering ATL with symptomatic skin lesions often progress to acute ATL. These patients even before progression are similar to those with cutaneous T-cell lymphoma, and are appropriate candidates for trials with monoclonal antibody, immunotoxin, and/or chemotherapy.

Table 1.A: Antitumor activity in LMB-2 patients who had major response:

Pt#	Dx	Dose mcg/ Kgx3	Best resp-onse	Dura-tion (mo)	Tumor location	% Neut-ralization 200ng/ml	Immuno-genicity limiting?
L215	HCL	30	PR	2	Blood	100% after C1	Yes
L217	CTCL	30,40	PR	7	Blood, erythroderma	100% after C6	Yes*
L226	CLL	50	PR	10	Blood, nodes	3% after C3	No
L230	HCL	63	CR	89	Spleen, nodes, blood	16% after C2	No
L232	HCL	63	PR	1	Blood	7% after C1	No
L233	HD	40	PR	2	Abdominal mass	0% after C1	No
L234	ATL	40	PR	13	Spleen, blood	100% after C2	Yes*
L235	HCL	40	PR	40	Blood	100% after C2	Yes*
CL03	CLL	40	PR	4	Blood	100% after C6	Yes*
HC02	HCL	40	PR	10	Blood	76% after C3	Yes

Patients numbers begin with L2 (phase I trial), CL (phase II CLL trial), and HC (phase II HCL trial). Duration indicates duration of major response.

Table 1.B: Antitumor activity in LMB-2 patients who had minor response:

Pt#	Dx	Dose mcg/ Kgx3	Best resp-onse	Activity and/or tumor location	% Neut-ralization 200ng/ml	Immuno-genicity limiting?
L206	MCL	6	MR	Pulmonary mass	2% after C1	No
L213	HD	30	MR	Abdominal mass	99% after C2	Yes*
L218	CLL	40	MR	Lymph nodes, spleen	0% after C1	No
L229	HD	50	MR	Pulmonary mass	100% after C1	Yes*
CL04	CLL	40	MR	Neck nodes	6% after C2	No
CL07	CLL	40	MR	Neck nodes	12% after C2	No
CL09	CLL	40,30	MR	Blood and nodes	100% after C6	Yes*
CT02	CTCL	30	MR	Improved skin, pruritis	100% after C2	Yes*
CT04	CTCL	30	MR	Skin lesions	100% after C2	Yes*

Patients numbers begin with L2 (phase I trial), CL (phase II CLL trial), and CT (phase II CTCL trial).

Table 1.C: Antitumor activity in LMB-2 patients with only transient response:

Pt#	Dx	Dose mcg/ Kgx3	Best resp-onse	Activity and/or tumor location	% Neut-ralization 200ng/ml	Immuno-genicity limiting?
L207	CLL	10	SD	↓ Spleen, CLL count	2% after C5	No
L216	ALCL	30	PD	↓ Cutaneous nodules	92% after C1	Yes
L219	LBCL	40	PD	↓ Cutaneous mass	65% after C1	Yes
L220	PTCL	40	SD	↓ Skin lesions	74% after C1	Yes
L224	ATL	50	PD	> 90% ↓ ATL cells	1% after C1	No
L225	CLL	50	SD	↓ CLL count	0% after C1	No
L231	HD	63	SD	↓ Neck mass	100% after C1	Yes*
L236	ATL	50	SD	> 90% ↓ leg mass	68% after C1	Yes
L238	CTCL	40	SD	↓ Skin lesions	100% after C2	Yes*
CL06	CLL	40	SD	↓ CLL count	1% after C6	No
CL11	CLL	40	SD	↓ CLL count	22% after C6	No
CL12	CLL	40	SD	↓ CLL count	45% after C1	Yes
CT01	CTCL	40	SD	↓ Itching/pain	3% after C1	No
CT03	CTCL	30	SD	Improved skin	100% after C1	Yes*
CT05	CTCL	30	SD	↓ Skin pain	100% after C1	Yes*

The first 2 characters of the patient number are L2 for the Phase I trial, CL for the phase II trial in CLL, CT for the phase II trial in CTCL, and HC for the phase II trial in HCL. Diagnoses (Dx) include mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), Hodgkin's disease (HD), hairy cell leukemia (HCL), anaplastic large cell lymphoma (ALCL), cutaneous T-cell lymphoma (CTCL), large B-cell lymphoma (LBCL), peripheral T-cell lymphoma (PTCL), and adult T-cell leukemia (ATL). Response abbreviations include complete remission (CR), partial response (PR), and marginal response (MR). Patients are coded for MR based on LMB-2 Phase I trial criteria, although later trials did not use MR. Percentages of neutralizing activity indicate the percent neutralization by serum of 200 ng/ml of LMB-2 against SP2/Tac cells, after the indicated cycle number. Yes* indicates which patients had > 75% neutralization of 1000 ng/ml of LMB-2, the criteria for ineligibility for retreatment.

1.3.7 Immunogenicity with LMB-2

As detailed in section **1.2.16.3**, immunogenicity is a major limiting factor in LMB-2 efficacy. Although only a few ATL patients have been treated, it is clear that immunogenicity is a potential problem in the efficacy of LMB-2 in that disease. This is expected given the significant rate of HAMA in ATL patients [12-14]. Thus, the enrollment of ATL patients after immunosuppressive treatment to reduce immunogenicity is reasonable, particularly since the FC employed for immunosuppression should also reduce tumor burden and thereby allow the LMB-2 to distribute to tumor cells more efficiently. If so, FC will also slow the regrowth of disease between cycles of ATL.

1.3.8 Drug supply and ability to administer 6 cycles of LMB-2

CTEP currently has enough LMB-2 to treat approximately 200 cycles on this protocol. Since the accrual ceiling is 37 patients but 4 of these would not be enrolled unless needed to replace patients who could not receive LMB-2, the average numbers of cycles of LMB-2 per patient is over 6. Moreover, based on average cycles/patient statistics on the phase II trials of CLL, CTCL and HCL, it is extremely likely that a large percentage of LMB-2 allocated to these trials will be available for ATL patients if needed.

1.3.9 Ability to achieve the accrual goals of the trial

The PIs in the Metabolism Branch who treat ATL patients are associate investigators on this trial, and ATL patients on this protocol will be treated at NIH in the hematologic malignancy section of the Medical Oncology Branch, as they are for ATL protocols directed by the Metabolism Branch. Several ATL trials which are still accruing patients, including the EPOCH-Rituximab protocol, and new ATL trials like the Siplizumab-EPOCH-R protocol, will not compete with this protocol since those trials are only for previously untreated ATL. It is anticipated that most patients on this FC/LMB-2 protocol will have had prior therapy, but the eligibility criteria will allow untreated cases in case such patients are ineligible for other trials. Other Metabolism Branch trials open for previously treated ATL include the alemtuzumab study, and the Ontak study. It is anticipated that the alemtuzumab study will complete accrual prior to approval of this study. The Ontak study should not compete with this trial since most patients spend only a few weeks on protocol and immunogenicity to Ontak would not result in antibodies against LMB-2. Approximately 1 new ATL patient per month is seen by the Metabolism Branch and it is estimated that 50-80 of these patients would be eligible either initially or eventually for this trial. Thus, it should be possible to meet the accrual goal of 29 patients over 3-4 years without requiring an increase in patient referrals.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Diagnosis of acute or lymphomatous ATL by flow cytometry of blood or immunohistochemistry of biopsy tissue, confirmed by NCI Laboratory of Pathology, and previously treated unless the patient is ineligible for or refuses other protocols or treatments for ATL.

2.1.1.2 Neutralizing antibodies less than or equal to 75% neutralization of 200 ng/ml of LMB-2.

2.1.1.3 At least 18 years old.

2.1.1.4 ECOG 0-2 (See **Appendix B** for definitions).

2.1.1.5 Able to understand and give informed consent.

2.1.1.6 Negative pregnancy test for females of childbearing potential.

2.1.1.7 The transaminases ALT and AST must each be ≤3-times the upper limits of normal (ULN) or ≤10-times normal if due to ATL. Albumin must be ≥3.0 gm/dL. Total bilirubin must be ≤ 1.5 mg/dL except in patients with Gilbert's syndrome (as defined by >80% unconjugated bilirubin) it must be <5mg/dl.

2.1.1.8 Creatinine < 2.0 mg/dL.

2.1.1.9 ANC ≥ 1000/uL and Platelets ≥ 50,000/uL.

2.1.1.10 Current or prior features of acute (corrected Ca++ >2.73 or LDH 2-fold above ULN) or chronic (LDH 1.5-2-fold above ULN or absolute lymphocyte count ≥4 $x10^9$/L with T-cells >3.5 $x10^9$/L) ATL. Patients with smoldering ATL (no acute or chronic features) and symptomatic ATL skin lesions are also eligible.

2.1.2 Exclusion Criteria

2.1.2.1 Prior therapy with LMB-2.

2.1.2.2 Central nervous system disease as evidence by clinical symptomatology.

2.1.2.3 Cytotoxic chemotherapy, steroids or Mab within 3 weeks of enrollment, except anti-Tac Mab (i.e. daclizumab) which cannot be used within 12 weeks of enrollment. Hydroxyurea is considered different from cytotoxic chemotherapy and may be used up to the day before enrollment providing it is not increased during the week prior to enrollment and that the patient's disease burden is not decreasing during that time.

2.1.2.4 Uncontrolled infection.

2.1.2.5 Untreated or uncontrolled 2nd malignancy.

2.1.2.6 Patients who are pregnant or breast-feeding (See section **2.2.6**).

2.1.2.7 Patients who have HIV or hepatitis C, since in these patients reductions in normal T- or B-cells would increase the risk of exacerbation of their underlying disease. Patients would not be excluded for hepatitis B surface antigen positivity if on Lamivudine or Entecavir.

2.1.2.8 Patients receiving warfarin (Coumadin®).

2.1.2.9 Patients with a left ventricular ejection fraction of < 45%.

2.1.2.10 Patients with a DLCO <50% of normal or an FEV1 <50% of normal.

2.1.2.11 No concomitant use of alternative complimentary therapies or OTC agents allowed without prior approval of the PI.

2.1.2.12 Tumor or lymph node masses > 4 cm.

2.1.3 Recruitment strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

2.2 SCREENING EVALUATION

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

The following activities will be performed only after the subject has signed the consent for this study for screening.

2.2.2 Anytime prior to enrollment: Immunohistochemistry (if flow cytometry negative) to detect CD25+ ATL cells in solid masses.

2.2.3 Within 2 months before enrollment: HIV, hepatitis B (Ag and core Ab), and C. CMV/EBV PCR (no further follow up required unless clinically indicated). Echocardiogram and Pulmonary Function Tests.

2.2.4 Within 28 days prior to enrollment: Lipid panel, fibrinogen, thrombin time, TSH, Free T3, Free T4, T3, T4, 24 hour urine for creatinine clearance, protein, and UPEP, Exercise Stress Test (ETT).

2.2.5 Within 2 weeks prior to enrollment: Physical exam. Flow cytometry to quantify circulating ATL cells, CD4+ and CD8+ lymphocytes, and normal B-cells. CT scan of chest, abdomen, pelvis, and neck, photography of skin lesions (per PI discretion), dermatology consult (per PI discretion), PET CT if needed to better define response (per PI discretion).

2.2.6 Pregnancy test (urine or blood) within 1 week before enrollment in women of childbearing potential. The effects of LMB-2 combined with FC on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation until 1 month after the last cycle of LMB-2.

2.2.7 Within 3 days before enrollment: CBC and diff, Chemistries (albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CK, chloride, CO2, creatinine, direct bilirubin, glucose, potassium, LDH, magnesium, sodium, phosphorus, total protein, uric acid), PT/PTT, urinalysis, IgA, IgG, IgM, GGT, haptoglobin, amylase, lipase and serum for tumor markers and neutralizing antibodies.

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 at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, and eligibility criteria.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a non-randomized trial of LMB-2 immediately following fludarabine-cyclophosphamide (FC), preceded by an initial cycle of FC alone. Patients without high levels of neutralizing antibodies (> 75% neutralization of 1000 ng/ml of LMB-2) may be retreated with FC/LMB-2 at minimum intervals of 13 days after FC alone and 20 days after FC with LMB-2. The following schema summarizes the timing of doses:

3.1.1 Fludarabine IV days 1-3 (F)

- Patients 1 -7, 10 – 14, and then ≥18: 25 mg/m^2/day
- Patients 8 – 9: 30 mg/m^2/day
- Patients 15-17: 20 mg/m^2/day

3.1.2 Cyclophosphamide IV days 1-3 (C)

- Patients 1 -7, 10 – 14, and then ≥18: 250 mg/m^2/day
- Patients 8 – 9: 300 mg/m^2/day
- Patients 15-17: 200 mg/m^2/day

3.1.3 LMB-2 QOD x3 doses, days 3, 5, and 7. Begin with 30 mcg/Kg·dose x3 doses. Escalate to 40 mcg/Kg·dose QOD x3 doses if 0/3 or 1/6 have DLT at 30 mcg/Kg·dose. Continue at 40 mcg/Kg·dose QOD x3 doses if 0-1 of 6 have DLT at 40 mcg/Kg·dose QOD x3 doses.

3.1.4 Repeat FC-LMB-2 for maximum of 6 cycles. The minimum interval is 13 days after cycle 1 and 20 days after cycles 2-6. Give the 1st cycle of FC without LMB-2 and give LMB-2 with cycles 2-7 of FC.

3.1.5 Response on the trial is based on pre-cycle 1 staging but patients will be restaged prior to cycle 2 to determine response to LMB-2.

3.1.6 Patients with CR or PD after FC alone will not receive LMB-2.

3.1.7 Accrual goals: Minimum 29, maximum 37.

3.1.8 Schema for timing of FC and LMB-2:

Cycle 1:

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day ≥ 14
FC	FC	FC					FC (next cycle)

Cycles 2-7:

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day ≥ 21
FC	FC	FC					FC (next cycle)
		LMB-2		LMB-2		LMB-2	

3.2 DRUG ADMINISTRATION

3.2.1 FC chemotherapy

3.2.1.1 Fludarabine by 30 minute i.v. infusion days 1 - 3

- Patients 1 -7, 10 – 14 and ≥18: 25 mg/m^2/day
- Patients 8 – 9: 30 mg/m^2/day
- Patients 15-17: 20 mg/m^2/day

3.2.1.2 Cyclophosphamide by 60 minute i.v. infusion days 1-3

- Patients 1 -7, 10 – 14 and ≥18: 250 mg/m^2/day
- Patients 8 – 9: 300 mg/m^2/day
- Patients 15-17: 200 mg/m^2/day

3.2.1.3 Total dose based on weight or BSA from last NIH measurement, measured within 1 week from each dose.

3.2.1.4 Prophylactic antiemetics may be given as necessary. Avoid prophylactic steroids, although up to 6 doses of 60 mg of i.v. solumedrol (or equivalent) may be used per cycle

to prevent or treat fever associated with LMB-2. Steroids in excess of this amount may be used to manage ATL as needed provided that the patient must be off systemic steroids (except to prevent adrenal insufficiency) for at least 6 weeks to be counted as a major responder.

3.2.1.5 Recommend at least 2L of water intake per day.

3.2.1.6 Filgrastim: After the first episode of grade > 2 neutropenia (ANC < 1000/uL), filgrastim will be administered at 300 mcg/day subcutaneously from day 5 to 1 day before the next cycle, and may be held when the ANC is > 5000/uL.

3.2.1.7 Example of drug administration days 1 and 2:

- Hour 0 - 1 500 ml of D5/0.45% NaCl
- Hour 1 - 2 Fludarabine 20-30 mg/m2·day iv over 30 minutes in 100 ml 0.9% NaCl
- Hour 2 - 3 Cyclophosphamide 200-300 mg/m2·day IV in 100 ml 0.9% NaCl over 60 minutes.
- Hour 3 - 4 500 ml of D5/0.45% NaCl over one hour

3.2.1.8 Example of drug administration day 3:

- Hour 0 - 1 500 ml of D5/0.45% NaCl
- Hour 1 - 2 Fludarabine 20-30 mg/m2 iv over 30 minutes in 100 ml 0.9% NaCl
- Hour 2 - 3 Cyclophosphamide 200-300 mg/m2 IV in 100 ml 0.9% NaCl over 60 minutes.
- Hours 3 - 5 1000 ml of D5/0.45% NaCl over two hours
- Hour 4 Hydroxyzine, ranitidine, and acetaminophen 1 hr before LMB-2
- Hour 5 - 5.5 LMB-2 iv over 30 min
- Hours 5.5 - 7.5 1000 ml of D5/0.45% NaCl over two hours
- Hour 10 Acetaminophen
- Hour 12 Hydroxyzine and ranitidine
- Hour 16 Acetaminophen
- Hour 22 Acetaminophen

3.2.1.9 Example of drug administration days 5 and 7:

- Hours 0 - 2 1000 ml of D5/0.45% NaCl over two hours
- Hour 1 Hydroxyzine, ranitidine, and acetaminophen 1 hr before LMB-2
- Hours 2 - 2.5 LMB-2 iv over 30 min
- Hours 2.5 - 4.5 1000 ml of D5/0.45% NaCl over two hours
- Hour 7 Acetaminophen
- Hour 10 Hydroxyzine and ranitidine
- Hour 13 Acetaminophen
- Hour 19 Acetaminophen

3.2.2 LMB-2

3.2.2.1 30-40 mcg/Kg·dose will be infused through a peripheral I.V. or central line in 50 ml of 0.9% NaCl and 0.2% albumin via a PAB container over 30 minutes every other day for 3 doses (QOD x 3). Additional IV fluid will be given as described below. FC and LMB-2 may be administered as an outpatient or inpatient, and the day 3 dose of LMB-2 should follow the day 3 doses of FC.

3.2.2.2 Premedication: Patients will be medicated with 25 mg hydroxyzine and 150 mg ranitidine orally 1 hour prior to and 8 hours after each dose. Acetaminophen 650 mg P.O. will be

given every 6 hours for 4 doses starting 1 hour prior to each LMB-2 dose. Emergency medications such as epinephrine and diphenhydramine should be available in the area where the patients will receive the LMB-2 infusion for treatment of an allergic reaction. Emergency equipment including oxygen should be available in the patient's room.

- I.V. Fluid: Patients will receive fluid prophylaxis, consisting of:
- 500 ml of D5/0.45% NaCl over 1 hours prior to cyclophosphamide.
- 500 ml of D5/0.45% NaCl over 1 hours after cyclophosphamide.
- 1000 ml of D5/0.45% NaCl over 2 hours prior to LMB-2
- 1000 ml of D5/0.45% NaCl over 2 hours after LMB-2
- On day 3, cyclophosphamide is given before LMB-2 and the post-hydration fluid for cyclophosphamide is replaced by the 1000 ml pre-hydration for LMB-2.
- Hydration may be prolonged at the discretion of the PI.

3.2.2.3 Vital signs of inpatients will be obtained at the beginning of infusion, at 15 minutes, & at the end of infusion, then every 60 minutes for 2 hours, then as per unit routine. Daily orthostatic blood pressure, weights, I/Os, and physical exam will be done. Daily orthostatic blood pressure will be measured after having the patient in supine and standing position for at least 2 minutes. If standing SBP decreases from supine SBP by > 20 mmHg, then another standing blood pressure measurement will be done at least 3 minutes after the first.

3.2.2.4 Vital signs of outpatients will be obtained at the beginning of infusion, at 15 minutes, & at the end of infusion, then every 60 minutes for 2 hours. Patients will be monitored for peripheral edema and orthostatic hypotension at least daily. Daily weights will also be recorded.

3.3 TREATMENT MODIFICATIONS

3.3.1 Definition of DLT

Grade III-V LMB-2 or FC-related toxicity, except:

3.3.1.1 Vascular leak syndrome (VLS): As specified by CTCAE 3.0 to be utilized until December 31, 2010, grade II VLS includes symptoms of fluid retention, including grade I-II weight gain. If a patient requires more than an hour's worth of hydration at 20 ml/Kg/hour for hypotension, then the patient will be considered to have grade III hypotension. Grade III hypotension in temporal association with VLS will also be considered grade III VLS. VLS resulting in respiratory compromise is considered grade III according to CTCAE 3.0 to be utilized until December 31, 2010. Respiratory compromise is defined as symptomatic pulmonary edema with at least grade III hypoxemia. Either grade $\geq$ III VLS or grade $\geq$ III hypotension is considered DLT.

Definition of Capillary Leak Syndrome (as stipulated by CTEP) to be utilized after January 1, 2011: As specified by CTCAE 4.0, grade II CLS is defined as symptomatic; medical intervention is indicated; this protocol further defines that if weight gain is the only feature of CLS in a patient, it will be considered a grade II CLS. If a patient requires more than an hour's worth of hydration of 20 ml/Kg/hour for hypotension, then the patient will be considered to have grade III hypotension. Grade III hypotension in temporal association with CLS will be consider also a grade III CLS. Respiratory compromise in the setting of CLS defined as symptomatic pulmonary edema requiring oxygen or > 10%

decrease in oxygen saturation will be consider a grade III CLS. Grade III hypotension or grade III CLS is dose limiting.

3.3.1.2 Alopecia is not considered DLT.

3.3.1.3 Grade II or III allergic reaction with asymptomatic bronchospasm or urticaria is considered DLT.

3.3.1.4 Grade III AST, ALT, GGT, and fever are not considered DLT. Fever and transaminase elevations are common with LMB-2 and have never been associated with poor hepatic function (i.e. hyperbilirubinemia, hyperammonemia). These guidelines are standard from immunotoxin trials.

3.3.1.5 Grade IV CPK associated with any other DLT or not resolving to < grade II within 2 weeks is considered DLT.

3.3.1.6 Hematologic toxicity is not considered DLT unless it fails to resolve to < grade 2 or baseline by day 18 after cycle 1 or after day 25 after cycles 2-7.

3.3.1.7 DLT from hepatotoxicity, CPK, and VLS is assumed from LMB-2, and hematologic toxicity from FC. Hemorrhagic cystitis is not expected at the 200-300 mg/m^2 dose level of cyclophosphamide.

3.3.1.8 Grade III proteinuria lasting < 2 weeks after the last dose of LMB-2 is not considered DLT, and needs to resolve to grade 0-2 prior to retreatment.

3.3.2 Modification based on previous cycle:

3.3.2.1 DLT on previous cycle at least possibly related to LMB-2 requires dose reduction of subsequent LMB-2 cycles by 10 mcg/Kg·dose QOD x3.

3.3.2.2 Lymphopenia and leukopenia are expected effects of the FC chemotherapy in order to prevent immunogenicity, and neutropenia down to 0.5 (grade 3) is also expected and modified by G-CSF, and these would not be considered toxicity requiring delay of retreatment or dose reduction.

3.3.2.3 Retreatment may be delayed up to 4 weeks to allow toxicity to resolve to grade 0-1 or to baseline. Prior to resolution of cytopenias, retreatment may resume at 50%, 25% and 0% of FC for grades 2, 3 and 4 thrombocytopenia, respectively, and 0% of FC for grade 4 neutropenia irrespective of the platelet count. Unless the cytopenias are related to a cause other than FC (i.e. sulfa or reversible viral infection), the doses of FC will not increase for subsequent cycles.

3.3.2.4 50% and 75% dose reductions (50% and 25% of original FC doses, respectively, are from Flinn et al., Blood, 96:71, 2000 [68].

3.3.2.5 For patients 1-7 and 10-14, who began with 25 mg/m^2 and 250 mg/m^2 dose levels of fludarabine and cyclophosphamide, respectively, a milder dose reduction was used for DLT [85], in which the 1st dose reduction required 25 mg/m^2 of fludarabine and 200 mg/m^2 of cyclophosphamide, and patients could be treated without FC if cytopenias did not resolve.

3.3.2.6 For patients 15-17, who began with 20 mg/m^2 and 200 mg/m^2 dose levels of fludarabine and cyclophosphamide, no dose reduction was needed due to lack of hematologic toxicity, and efficacy particularly against leukemic ATL was unsatisfactory.

3.3.2.7 Current dose modifications for chemotherapy

	Fludarabine	Cyclophosphamide	Indication
Current starting doses	25 mg/m^2·day days 1-3	250 mg/m^2·day days 1-3	
50% level (50% reduced)	12.5 mg/m^2·day days 1-3	125 mg/m^2·day days 1-3	Grade 2 platelets
25% level (75% reduced)	6.25 mg/m^2·day days 1-3	62.5 mg/m^2·day days 1-3	Grade 3 platelets
0% level (100% reduced)	0	0	Grade 4 platelets or ANC

3.3.2.8 Dose modifications for LMB-2 DLT:

	LMB-2
1st dose reduction	10 mcg/Kg·dose x3 doses less than enrollment dose
2nd dose reduction	20 mcg/Kg·dose x3 doses less than enrollment dose
3rd dose reduction	Off treatment

3.3.3 Modification based on current cycle:

3.3.3.1 Cancel remaining doses of cycle if DLT during the cycle.

3.3.3.2 May delay dosing up to 48 hr if toxicity less than DLT.

3.4 PHARMACOKINETIC STUDIES (LMB-2 ONLY)

3.4.1 Procedures

Blood samples will be drawn by the nurse at the times outlined below. Tubes must be labeled with the patient's name, medical record number, date of birth, date, time drawn, and time related to LMB-2 end of infusion. PKs should not be drawn from the line which was infusing LMB-2. Since PKs are not drawn until LMB-2 finishes infusing, it is permissible for PKs to be drawn out of a different port of the same catheter that is used to infuse the LMB-2. Samples of 2 ml of blood will be drawn in a 6 ml sodium heparin tube (green top) using a syringe or Vacutainer. Tubes of blood collected at the clinical center (including both inpatient and outpatient units) should be stored upright in the "Kreitman" container in the refrigerator in the inpatient unit. These samples will be collected daily Monday through Friday and taken to our contract lab at Frederick National Laboratory for Cancer Research: David Waters, PhD, Building 560, Lab 11-05, 1050 Boyles St, Frederick, MD 21702, Phone: 301-846-5831.

3.4.2 Day 3 (day 1 of LMB-2)

Pre, and then 2 min, 1, 2, 3, 4, 10 hours after end of infusion, and next morning.

3.4.3 Day 5 (Day 3 of LMB-2)

Pre and then 2 min, and next morning.

3.4.4 Day 7 (Day 5 LMB-2)

Pre and then 2 min, 1, 2, 3, 4, and 10 hours after end of infusion, and next morning.

3.4.5 Acceptable error in PK time points:

When possible, +/- 2 min for 2 min, +/- 30 min for later samples. The next morning sample may be combined with the morning lab draw, and may be combined with the 10 hour time point if that time point is within 2 hours of the morning blood draw. The actual time of sample collection should be recorded.

3.5 PROTOCOL EVALUATION

3.5.1 Prior to each cycle (do precycle tests within 3 days prior to cycle, i.e. day 11 of cycle 2 or day 18 of cycles 3-7):

Labs: CBC, diff, chemistries (Albumin, alkaline phosphatase, ALT, amylase, AST, bilirubin, BUN, calcium, CK, chloride, CO2, creatinine, direct bilirubin, GGT, glucose, potassium, LDH, lipase, magnesium, sodium, phosphorus, total protein, uric acid), PT/PTT, urinalysis, IgA, IgG, IgM, haptoglobin, beta-2-microglobulin, serum for sCD25 and other tumor markers, and neutralizing antibodies, CRP, flow cytometry

3.5.1.1 Chest X-ray (if no Chest CT obtained)

3.5.1.2 EKG

3.5.1.3 CT or other imaging of relevant level based on known disease burden, if needed to determine response.

3.5.1.4 Echocardiogram

3.5.2 On days of LMB-2 dosing:

3.5.2.1 Labs: CBC, diff, Chem-20 (Albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CK, chloride, CO2, creatinine, direct bilirubin, glucose, potassium, LDH, magnesium, sodium, phosphorus, total protein, uric acid), urinalysis

3.5.2.2 On day 3 (day 1 of LMB-2 dosing): beta-2-microglobulin, serum for sCD25 and other markers, CRP

3.5.2.3 On day 5 (day 3 of LMB-2 dosing): EKG 10-30 min after end of infusion (time of maximum expected LMB-2 concentration).

3.5.2.4 On day 7 (day 5 of LMB-2 dosing): EKG before dosing.

3.5.3 After LMB-2 dosing (days 8-11 or days 6-9 of LMB-2):

3.5.3.1 Labs: CBC, diff, Chem-20, urinalysis, PT/PTT, IgA, IgG, IgM, haptoglobin, amylase, lipase, beta-2-microglobulin, serum for sCD25 and other tumor markers, and neutralizing antibodies, CRP, flow cytometry

3.5.3.2 Chest X-ray

3.5.4 Initial optional studies (at PI discretion):

3.5.4.1 MRI if needed to better define response

3.5.4.2 PET CT if clinically indicated to better define response. Cannot be used for response assessment but may be used to determine if masses on CT are evaluable. PET scans if clinically indicated are usually performed every 6 weeks during pre-cycle staging

3.5.4.3 Bone marrow biopsy & aspirate if needed to define response

3.5.4.4 Photography of skin lesions.

3.5.4.5 Blood for lymphocyte collection for cytotoxicity assays

3.5.4.6 Blood for ELISA assays to correlate with immunogenicity assays

3.5.4.7 Skin biopsy

3.5.4.8 HLA typing to correlate with immunogenicity assays

3.5.4.9 Fine needle aspirates (FNA) of additional sites to determine antigen positivity and assess tumor markers or capillary permeability at the tumor site. FNA of accessible masses by pathologists in NCI cytopathology typically requires a 25 gauge needle and up to 3 passes for adequate material for cytopathology and flow cytometry.

3.5.5 At follow-up, tests at discretion of PI, at up to 6-month intervals

Physical exam, & if relevant, dermatology consult, clinical photography, flow cytometry, imaging of tumor sites, CBC + diff, chem-20, urinalysis, PT, PTT, IgA, IgG, IgM, GGT, haptoglobin, amylase, lipase, sCD25 (& other tumor markers), neutralizing antibodies, CRP, and beta-2-microglobulin (see Section **14.1**).

3.6 CONCURRENT THERAPIES

3.6.1 Listed in Section **3.1**

3.7 RADIATION THERAPY GUIDELINES

Therapeutic radiation may be undertaken to a localized area as long as there is measurable disease outside the radiation port and the area rated is not considered a risk for worse immunosuppression. Areas treated with radiotherapy will not be used in response assessment.

3.8 COST AND COMPENSATION

3.8.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by an insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.8.2 Compensation

Participants will not be compensated on this study.

3.8.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.9 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

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.

3.9.1 Off Treatment Criteria: (No treatment but data can be obtained)

- Progressive disease unless it occurs during a dosing delay for toxicity as specified by protocol Section **3.3.3.2**.
- > 75% neutralization of 1000 ng/ml of LMB-2.
- Grade III Allergic Reaction and grade II urticaria despite premedication.
- More than 2 dose reductions of LMB -2.
- Grade IV DLT other than ALT, AST, GGT, CPK.
- Intercurrent illness or medical circumstances.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

3.9.2 Off-Study Criteria

- Screen failure
- Patient decides to withdraw from the study
- Completed study follow-up period
- Death

3.10 POST STUDY EVALUATION (FOLLOW-UP)

May repeat baseline testing at up to 6-month intervals as deemed necessary by the PI to follow response status and toxicity.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 SUPPORTIVE CARE

4.1.1 Allergic reaction

Treated acutely with antihistamines (including diphenhydramine, hydroxyzine, & ranitidine), fluids, bronchodilators, and/or epinephrine.

4.1.2 Nausea and Vomiting

Patients who develop nausea will be treated with a serotonin 5-HT_3 receptor inhibitor for at least 24 hours after their last episode of nausea. Other antiemetics such as prochlorperazine, metoclopramide, or lorazepam may be used in addition if necessary.

4.1.3 Myalgias

Patients who develop myalgias may be given acetaminophen 650 to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2. It may then be given as needed. Patients may receive opioid analgesics if acetaminophen is inadequate.

4.1.4 Vascular/Capillary leak syndrome

Supportive care may include fluid and electrolyte management, diuresis, albumin, and cardiovascular support.

4.1.5 Hypotension

Patient will be encouraged to increase oral fluid intake. In addition, for an orthostatic SBP change of >20 mm Hg and an absolute SBP of <100 mm Hg, an IVF bolus may be given as deemed clinically appropriate. Refractory hypotension may require treatment in the intensive care unit with pressors.

4.1.6 Fever

Patients who develop temperatures >38.0° C may receive scheduled acetaminophen 650 to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2. It may then be given as needed.

4.1.7 Thrombocytopenia

Should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should only be given for a platelet count below 10,000/uL. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count > 50,000/uL.

4.1.8 Symptomatic anemia

Should be treated with appropriate red blood cell support. Transfusion is recommended if the hemoglobin falls below 8g/dL. Recombinant erythropoietin may be also used.

4.1.9 Febrile Neutropenia

Life-threatening complication requiring hospitalization and urgent broad-spectrum antibiotics. Hematopoietic growth factors may be used if clinically indicated. Such cases will be evaluated individually to determine the toxicity grade. Neutropenia due to LMB-2 is not expected.

4.1.10 Central venous access devices

Such as a temporary internal jugular or subclavian lines, PICC lines, semi-permanent HICKMAN, Groshong catheters, or medi-port implanted devices can all be used in this study. All devices will have nursing supervision and include patient self-care instruction.

4.2 NUTRITIONAL ASSESSMENT AND PSYCHOLOGICAL SUPPORT

Refractory neoplasms are commonly complicated by malnutrition. Patients with weight loss or evidence of wasting syndrome should have a nutritional consult. When necessary, social work will be proactively involved with these patients' psychosocial well-being.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR 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. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after removal from study treatment or until off-study, whichever comes first.

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.
- A pre-existing (baseline) laboratory abnormality will be considered the last one obtained prior to the first dose of drug, unless the PI considers an abnormality of higher grade occurring within 100 days prior to the first dose to be a truer baseline.
- Each AE will be reported with an onset date when it begins above baseline, a resolution date as the last time during a cycle that it resolves to or below baseline, and the maximum grade achieved. Thus, the same AE might recur after each cycle, but would not be reported multiple times per cycle unless it is judged by the PI to be significantly different in its attribution.

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

5.2 RESPONSE CRITERIA

5.2.1 Response criteria are based on the International Workshop to Standardize Response Criteria for non-Hodgkin's Lymphoma.

5.2.2 Duration. A response must last for at least four weeks to be considered a major response, but > 8 weeks to meet the primary endpoint of the study.

5.2.3 Complete Remission (CR). Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease related symptoms if present before therapy and normalization of those biochemical abnormalities (for example LDH) definitely assignable to the lymphoma. All lymph nodes must have regressed to normal size (less than or equal to 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in greatest diameter must have decreased to less than or equal to 1 cm or by more than 75 percent in the sum of the products of the greatest diameters. The spleen, if considered to be enlarged before therapy, must have regressed in size and not be palpable on physical examination. The bone marrow must show no evidence of disease by histology, and need not be repeated if otherwise in CR. Peripheral blood must show a normal pattern by flow cytometry to qualify for a complete remission. Molecular studies will be used to determine response. Because of the limitation in performing serial PET scans and difficulty in verifying that PET abnormalities indicate malignant disease, they will not be used to determine response.

5.2.4 **Complete response unconfirmed (CRu).** As per complete remission criterion except that if a residual node is greater than 1.5 cm, it must have decreased by greater than 75 percent in the sum of the products of the perpendicular diameters. Lymphocyte aggregates within

the bone marrow must be negative for T-cell markers characteristic of adult T-cell leukemia lymphoma.

5.2.5 **Partial response (PR)**. Reduction by ≥ 50% of leukemia cell count or ≥ 50% reduction in the size of all measurable lesions, and no increase in size of any measurable or evaluable lesion or appearance of new lesion. Flow cytometry will not prevent consideration of PR or determine relapse from PR if the ATL count by flow cytometry remains below 100 cells/mm^3 and the patient qualifies for PR by other parameters.

5.2.6 **Stable disease (SD).** Neither a response nor progressive disease.

5.2.7 **Progressive disease (PD).** Appearance of new lesions, or an increase of 50% or greater in the sum of the product of the perpendicular diameters of the measurable lesions or persistent (at least two determinations) doubling of the peripheral blood leukemic cell count.

5.2.8 **PET scanning.** Because PET scanning may not be obtained on every cycle and may not be routinely obtainable at baseline, it may not be used for response assessment.

5.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized until December 31, 2010 for AE reporting. CTCAE version 4.0 will be utilized beginning January 1, 2011. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

6 STATISTICAL CONSIDERATIONS

6.1 STUDY DESIGN/ENDPOINTS

The primary objective of this study is to determine, in a nonrandomized pilot fashion, if after verifying its safety, the use of fludarabine and cyclophosphamide (FC) prior to LMB2 in patients with ATL is able to result in a minimally durable clinical response rate and an immunogenicity rate which may be an improvement over that demonstrated previously from CAMPATH.

6.2 DOSE-ESCALATION PHASE

In order to establish the safety of the proposed regimen, initially a small number of patients will be treated at 30 mcg/Kg·dose x3 doses prior to evaluating the intended dose of 40 mcg/Kg·dose x3 doses as follows. Initially, three patients will be enrolled and treated with LMB-2 at 30 mcg/Kg·dose x3 doses after receiving FC days 1-3. If 0/3 experience DLT at this level, then 3 patients will be enrolled at 40 mcg/Kg·dose x3 doses. On the other hand, if 1/3 have DLT at 30 mcg/Kg·dose x3 doses, then 3 more patients will be added at 30 mcg/Kg·dose x3 doses level and if 1/6 total have DLT, then accrual will proceed to 40 mcg/Kg·dose x3 doses. If2 of 3-6 patients are noted to have a DLT at 30 mcg/Kg·dose x3 doses, then accrual to the trial will end. If 0/3 experience DLT at 40 mcg/Kg·dose x3 doses, then 3 more patients will be enrolled at 40 mcg/Kg·dose x3 doses, and if 0/6 or 1/6 have DLT, then 40 mcg/Kg·dose x3 doses will be the full treating dose for the phase II portion of the trial. On the other hand, if 1/3 have DLT at 40 mcg/Kg·dose x3 doses then 3 more patients will be added at this level, and if 1/6 total have DLT, then 40 mcg/Kg·dose x3 doses will be the full treating dose for the phase II portion. However, if 2 patients have DLT at 40 mcg/Kg·dose x3 doses, then 30 mcg/Kg·dose x3 doses will become the

full treating dose after demonstrating that 6 patients have been treated at 30 mcg/Kg·dose x3 doses and no more than 1/6 have a DLT.

6.3 METHOD FOR EFFICACY DETERMINATION

In the previous trial with CAMPATH alone, there were a total of 10 clinical responses in 23 evaluable patients, and 7 of the 23 responses lasted for at least 8 weeks (30.4% minimally durable responses). It would be of interest to determine if in the present trial, the combination of agents would be able to result in a proportion of patients with responses lasting longer than 8 weeks which is more than previously identified. Since the 30% rate has its own confidence interval associated with it, the objective of the present trial will be to conduct a phase II study using a two-stage Simon optimal design which will rule out a 35% rate of patients having responses lasting > 8 weeks, and which will target a 60% rate of this outcome. To do so, using alpha=0.10 and beta=0.10, the trial will initially enroll 16 evaluable patients (which include patients from the phase I portion of the trial who were treated at the phase II dose level) and if 0 to 6 have PR or CR lasting over 8 weeks, then no further patients will be accrued. A pause in the accrual may be necessary to determine if this is the case prior to beginning to enroll to the second stage. If 7 or more patients in the 16 evaluable patients have responses lasting over 8 weeks, then accrual will continue until a total of 27 evaluable patients have been enrolled. If 7 to 12 of 27 have responses lasting over 8 weeks, this will be considered to be unsatisfactory, while if there are at least 13 patients of 27 with responses lasting over 8 weeks, this will be considered sufficiently promising for further development. Under the null hypothesis (35% proportion with responses lasting > 8 weeks), the probability of early termination is 69%.

6.4 IMMUNOGENICITY

As well, the rate of immunogenicity (defined as > 25% neutralization of 1000 ng/ml of LMB-2) is an important endpoint for the trial. The previous fraction of all non-CLL patients treated with LMB2 experiencing this degree of immunogenicity is 29/48 (60.4%). It would be desirable to be able to demonstrate if the treatment in the current trial is associated with a lower rate of immunogenicity than suggested by this group of historical controls. As one type of evaluation, if we enroll 27 evaluable patients on this trial, and if the proportion with immunogenicity were 30% (a 30% reduction), there would be 85% power to identify this difference as being lower than controls, with a one-tailed 0.10 Fisher's exact test. This would provide at least modest evidence that a reasonable level of improvement was associated with this treatment. As an alternative interpretation, if there are 12 or fewer of 27 evaluable patients with immunogenicity, then the probability of observing this number is 96.4% if the true rate of immunogenicity were 30%. It would be 7.4% if the true rate was 60%. Finally, the one sided 95% upper confidence interval bound on 12 of 27 is 62% and the one sided 90% upper confidence interval bound on 12 of 27 is 58%. Thus, observing 9 patients of 27 with immunogenicity would allow us to state that there is a trend toward a difference compared to 29 of 48, and observing 12 or fewer of 27 with immunogenicity would suggest that the rate was lower than 60% if this were to be interpreted as a fixed parameter. Since observing > 12 patients with immunogenicity any time during accrual would indicate consistency with the previously noted 60% immunogenicity rate, or higher, we would plan to stop accrual to the trial if a 13th patient with immunogenicity were noted any time during the phase II portion of the trial. Patients at all dose levels of LMB-2 and FC are evaluable for early stop with respect to immunogenicity.

6.5 COMPARING PFS WITH ALEMTUZUMAB STUDY

As a secondary evaluation, a comparison of Kaplan-Meier curves from the prior CAMPATH-only trial and the present one will be done using a log-rank test, with the recognition that the comparison will be done using the limited number of patients available and the associated imprecision associated with it.

6.6 COMPLETE REMISSION

It should be noted that if patients experience a complete response to FC with no evaluable disease remaining, then they will not be offered LMB-2 and these patients will be replaced in the phase II evaluation with other patients. It is expected that no more than 4 such patients will need to be replaced. These patients will not be included in the PFS curve or evaluation but will be described when the study is published.

6.7 SECONDARY OBJECTIVES

Other secondary objectives will be evaluated using standard statistical techniques such as correlation coefficients, trend tests, and multi-group comparisons, either with parametric methods or non-parametric depending on the distributions of values obtained. These evaluations include describing how blood levels of LMB-2 (AUC, Cmax) are related to toxicity and response; describing how the development of neutralizing antibodies affects blood levels of LMB-2 and toxicity; and describing how soluble Tac-peptide (sIL2Ra) levels are associated with response to treatment with LMB-2. Since these evaluations will all be considered secondary, the results will be presented using unadjusted p-values and will be accompanied by an explanation that the evaluations were secondary and hypothesis generating.

6.8 ACCRUAL

It is expected that 1-2 patients per month can be recruited for enrollment onto this trial. Allowing for up to 6 patients on a lower dose than used for the phase II evaluation, 27 patients in the phase II evaluation, and then up to 4 patients who may be replaced if they are not offered LMB-2 or non-evaluable for some other reason, a total of 37 patients may be required to be accrued onto the trial. It is expected that 2-3 years is a reasonable time frame in which to accrue all needed subjects. The accrual ceiling will be 48 to account for inevaluable participants.

7 HUMAN SUBJECTS PROTECTIONS

7.1 RATIONALE FOR SUBJECT SELECTION

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in any one patient group. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of patients exposed to a potentially toxic treatment on the one hand and the need to explore gender and ethnic aspects of clinical research on the other. If differences in the outcome which correlate with gender or ethnic identity are noted, accrual may be expanded, or a follow-up study may be written to investigate these differences.

7.2 INCLUSION OF WOMEN AND MINORITIES.

Both men and women and members of all races and ethnic groups are eligible for this trial.

7.3 PARTICIPATION OF CHILDREN

Only patients 18 years of age or older will be enrolled on this study, since the safety of this agent has not been previously defined in a pediatric population.

7.4 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 **7.5**), 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 to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **7.9.1** for consent procedure.

7.5 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients will receive evaluation and treatment of their tumor at the National Cancer Institute's Clinical Center. This protocol may or may not benefit an individual, but the results may help the investigators learn more about the disease and develop new treatments for patients with this disease. Benefit cannot be promised, nor can the chance of benefit be accurately predicted. This research treatment is unlikely to be curative but may offer temporary control of the disease. The disease eligible for this protocol is considered incurable. Patients will generally have a poor prognosis and have no standard options known to significantly improve survival.
The risks and benefits of participation for adults who become unable to consent, are no different than those described for patients who are less vulnerable.

7.6 ALTERNATIVE APPROACHES OR TREATMENTS

Patients will be consented verbally and in writing regarding the risks and benefits of this trial, the treatment requirements, and alternative approaches to entering on this trial.

7.7 PROCEDURES FOR PROTECTING AGAINST OR MINIMIZING ANY POTENTIAL RISKS

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients which are currently unforeseeable. Patients will be examined and evaluated prior to enrollment and prior to each cycle. The Clinical Center staff will observe all patients during the drug administration. All evaluations to monitor the treatment of patients will be recorded in the patient chart. Patients are required to have a local physician to provide long-term care and to monitor for complications. They will have blood draws at home to monitor side effects. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

7.7.1 Identified Risks to Study Procedures

7.7.1.1 Blood sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. Up to 45 mL of research blood may be collected at any follow-up visit.

7.7.1.2 Urine Collection

No physical risks are associated with urine collection.

7.7.1.3 Clinical Photography

No physical risks are associated with clinical photography.

7.7.1.4 Ultrasound

No physical risks are associated with ultrasound procedures.

7.7.1.5 Imaging/scans

In addition to the radiation risks discussed below, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

7.7.1.6 MR Imaging

The risks of MR imaging are relatively small.

The US Food and Drug Administration has issued warnings that administration of gadolinium (updated September 9, 2010), the MRI contrast imaging agent used in this protocol, has been associated with development of a disease called **nephrogenic systemic fibrosis (NSF)**. The syndrome is rare (approximately 600 cases reported worldwide as of September 2010, out of several million administrations of gadolinium), but disabling and in some cases, fatal. All cases to date have occurred in patients with severe renal disease, including patients on dialysis. NSF has been nearly eradicated secondary to careful screening of renal function and avoiding use of gadolinium in patients with eGFR <30 ml/min/1.73 BSA. Even in patients with end stage renal disease, there have been only rare occurrences of NSF because of precautions taken to use more stable contrast agents at lower doses. This protocol excludes patients with severe renal insufficiency (eGFR <30 ml/min/1.73 BSA). The FDA has issued warnings in 2017 and 2018 that some gadolinium may be retained in the brain, bone and skin although health risks of accumulation have not been reported to date. In accordance with the FDA Drug Safety Communication of 05/16/2018, the Medication Guide for gadobutrol (or other macrocyclic gadolinium contrast agent if applicable) will be made available to all subjects with scans that will involve gadolinium-based contrast agent administration.

7.7.1.7 Risks from Radiation Exposure

On this study, during follow-up, participants may receive up to 2 CT and 2 PET/CT scans per year. The total radiation dose for research purposes will be approximately 5 rem. The risk of getting cancer from the radiation exposure in this study is 0.5% and of getting a fatal cancer is 0.3%.

7.8 PROVISIONS FOR MONITORING DATA COLLECTION TO ENSURE SAFETY OF SUBJECTS

As information is gathered from this trial, clinical results will be shared with patients while maintaining patient confidentiality. Laboratory and clinical data will be frequently gathered, and any new significant findings found during the course of the research, which may affect a patient's willingness to participate further, will be explained. Moreover, in all publications and presentations resulting from this trial, patients' anonymity will be protected to the maximum extent possible. Authorized personnel from the National Cancer Institute (NCI) and Food and Drug Administration (FDA) may have access to research files in order to verify that patients' rights have been safeguarded. In addition, patient names will be given to the Central Registration Office to register and verify patients' eligibility.

7.9 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable 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 used in compliance with policy, including HRPP Policy 303) 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.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

<u>Manual (non-electronic) signature on electronic document</u>:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

7.9.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **7.4**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **7.9**.

8 CORRELATIVE STUDIES FOR RESEARCH

8.1 BIOSPECIMEN COLLECTION

8.1.1 Blood, bone marrow, lymph node, skin, and other tumor samples.

Research being conducted: Malignant cells may be stored to determine sensitivity to LMB-2 or to related agents. T-cell receptors may be cloned to serve as sensitive indicators of minimal residual disease, and serum markers for disease may also be determined.

Samples to be collected:

- Soluble CD25, and other tumor markers: To estimate the amount of cancer cells in the body by measuring proteins which fall off cancer cells and go into the blood. Requires about 1 teaspoon. Soluble CD25 can also be measured on tumor tissue, including lymph node or skin biopsies which can be done non-invasively or are performed for diagnosis.
- Skin biopsy: To determine whether skin lesions have ATL cells, and if so, to determine the extent to which ATL cells are cleared with FC or FC/LMB2.
- HLA typing to better understand the immune system in patients getting LMB-2. Requires about 1 teaspoon.
- PAX-gene tube: To obtain RNA to study tumor markers, and an assay called micro-arrays, to study why some patients do not respond as well as others to LMB-2. Candidate genes to study would be those mediating apoptosis and cytokine release. Requires about 1/2 teaspoon.
- DNA samples to look for abnormalities which might make a patient more susceptible to toxicity. Candidate genes to study would be those mediating apoptosis and cytokine release. Requires about 1/2 teaspoon.
- Samples to determine levels of LMB-2 in the blood, urine, and other tissues by activity or immuno (ELISA) assays.
- Flow cytometry assays to quantify tumor markers on the malignant cells. Requires about 1/2 tablespoon.

- Cytotoxicity assays. Leukemia or lymphoma cells from the blood, bone marrow, or other tissues may be tested with LMB-2 and related drugs to determine if the malignant cells can be killed outside the body. Requires 1-3 tablespoons. For follow-up studies in patients off-treatment, which will not affect eligibility for enrollment or retreatment, a non-radioactive neutralization assay may be used, under non-CLIA conditions. For additional correlation between the 2 assays, serum samples are saved to enable the new assay to be run with historical samples previously tested using the radioactive CLIA assay.

Assays which would have significant risk to the patient, including studies of genetic cancer susceptibility, will not be done.

Note: tubes and media for research tests may be substituted based on availability with the permission of the PI or laboratory investigator

8.2 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.

8.2.1 Timeframe and location of storage:

Samples will be stored and cataloged longer than a year, in alarmed freezers at our Leidos Biomedical Research, Inc. contract lab in Frederick, MD where neutralizing antibodies and PK samples are tested. The contact information is: David Waters, PhD, Building 560, Lab 11-05, 1050 Boyles St, Frederick, MD 21702, Phone: 301-846-5831. Portions of samples which are stored at Frederick National Laboratory for Cancer Research may also be stored and tested in the LMB lab (Building 37) for longer than a year providing there is sample remaining after studies are done. All samples will be stored with unique patient numbers and without personal identifiers. After closure of the protocol, the samples will either be destroyed, or their storage and use will be governed by a subsequent protocol. Samples at Frederick National Laboratory for Cancer Research will be tracked in a secure electronic database and the PI will report destroyed samples per the requirements of Section **9.2** if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent.

9 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

9.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

9.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

9.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-

800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

9.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

9.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

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

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

9.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

9.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis (every 1-2 weeks) 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 **9.2.1** 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.

- All serious adverse events must be reported immediately by telephone to the Principal Investigator, Dr. Robert Kreitman (301-480-6187). Call 301-496-1211 after hours.
- A summary of the completed study will be submitted to IDB/CTEP within 2 months of study completion. A status report will be submitted and presented at upcoming NCI meetings as requested.

10 SPONSOR PROTOCOL/SAFETY REPORTING

10.1.1 Phase 2 and 3 Adverse Event Reporting Table

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5	Grades 4 & 5
	Unexpected and Expected	Unex-pected	Expected	Unexpected		Expected		Unex-pected	Expected
				with Hospitali-zation	without Hospitali- zation	with Hospitali- zation	without Hospitali- zation		
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

1 Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:
CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:
· Grade 4 and Grade 5 unexpected events
CTEP-AERS 10 calendar day report:
· Grade 3 unexpected events with hospitalization or prolongation of hospitalization
· Grade 5 expected events
2 Although a CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.
December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

10.1.2 Comprehensive Adverse Events and Potential Risks list (CAEPR) for LMB-2 (anti-Tac (114)-PE-38, NSC 676422)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. 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 via CTEP-AERS (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 LMB-2 (Anti-Tac (114)-PE-38).

> **NOTE**: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 1.3, July 26, 2013[1]

Adverse Events with Possible Relationship to LMB-2 (Anti-Tac[114]-PE-38) (CTCAE 4.0 Term)	**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	
Anemia	
CARDIAC DISORDERS	
Left ventricular systolic dysfunction	***Left ventricular systolic dysfunction (Gr 2)***
Pericardial effusion	***Pericardial effusion (Gr 2)***
Sinus tachycardia	
GASTROINTESTINAL DISORDERS	
Abdominal distension	
Diarrhea	
Nausea	***Nausea (Gr 2)***
Vomiting	***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	
Edema face	***Edema face (Gr 2)***
Edema limbs	***Edema limbs (Gr 2)***
Fatigue	***Fatigue (Gr 2)***
Fever	***Fever (Gr 2)***
IMMUNE SYSTEM DISORDERS	
Allergic reaction	***Allergic reaction (Gr 2)***
INVESTIGATIONS	
Alanine aminotransferase increased	***Alanine aminotransferase increased (Gr 2)***
Alkaline phosphatase increased	
Aspartate aminotransferase increased	***Aspartate aminotransferase increased (Gr 2)***
CPK increased	
Creatinine increased	***Creatinine increased (Gr 2)***
GGT increased	
Platelet count decreased	***Platelet count decreased (Gr 2)***
Weight gain	***Weight gain (Gr 2)***
METABOLISM AND NUTRITION DISORDERS	
Anorexia	
Hypoalbuminemia	***Hypoalbuminemia (Gr 2)***

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	
Back pain	
Chest wall pain	
Myalgia	***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS	
Dizziness	
Headache	***Headache (Gr 2)***
RENAL AND URINARY DISORDERS	
Hematuria	***Hematuria (Gr 2)***
Proteinuria	***Proteinuria (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	
Dyspnea	
Pleural effusion	
VASCULAR DISORDERS	
Capillary leak syndrome	***Capillary leak syndrome (Gr 2)***
Hypotension	***Hypotension (Gr 2)***

[1]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.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[3]Muscle weakness includes Generalized muscle weakness, Muscle weakness left-sided, Muscle weakness lower limb, Muscle weakness right-sided, Muscle weakness trunk, and Muscle weakness upper limb under the MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS SOC.

Also reported on LMB-2 (Anti-Tac[114]-PE-38) trials but with the relationship to LMB-2 (Anti-Tac[114]-PE-38) still undetermined:

CARDIAC DISORDERS - Acute coronary syndrome; Myocardial infarction; Paroxysmal atrial tachycardia; Restrictive cardiomyopathy; Supraventricular tachycardia
EYE DISORDERS - Blurred vision; Eye pain
GASTROINTESTINAL DISORDERS - Dyspepsia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Non-cardiac chest pain
IMMUNE SYSTEM DISORDERS - Anaphylaxis
INFECTIONS AND INFESTATIONS – Infection[2]
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall
INVESTIGATIONS - Blood bilirubin increased; Cardiac troponin I increased; Fibrinogen decreased; Weight loss

METABOLISM AND NUTRITION DISORDERS - Hypokalemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Muscle weakness[3]; Musculoskeletal and connective tissue disorder - Other (acute rhabdomyolysis); Neck pain; Pain in extremity
NERVOUS SYSTEM DISORDERS - Dysgeusia; Presyncope; Syncope; Vasovagal reaction
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Pneumonitis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Rash maculo-papular
VASCULAR DISORDERS - Hypertension

Animal Data: The following toxicities have been observed in animal studies with LMB-2 (Anti-Tac[114]-PE-38):
leukocytosis

Note: LMB-2 (Anti-Tac[**114**]-PE-38) 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.

- Expedited AE reporting timelines defined:
  "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution with the exception of events listed in Section **3.3.1** (Definition of DLT).
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.
- An expedited AE report for all protocols utilizing agents under a CTEP IND must be submitted electronically to CTEP via CTEP-AERS.
- Adverse events that fulfill the 24-hour reporting requirement must be reported electronically via CTEP-AERS at http://ctep.cancer.gov. In the rare event when Internet connectivity is disrupted, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, a 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.
- Templates must be FAXED to CTEP at 301-230-0159
- When internet connectivity is restored, a report submitted on a paper template must be entered into electronic CTEP-AERS by the original submitter of the report at the site. All expedited AE reports must also be sent to the local IRB according to local IRB policy and procedure.

- All AEs reported via CTEP-AERS must also be reported via the routine AEs reporting defined by the protocol.

10.2 Expected Adverse Events

10.2.1 **Grade 4**: none

10.2.2 **Grade 3**: AST, SGOT, ALT, SGPT, GGT, Albumin, serum-low (hypoalbuminemia), platelets.

10.2.3 Grade 2:

10.2.3.1 Blood Bone Marrow: platelets, hemoglobin, Neutrophils/granulocytes (ANC/AGC), Leukocytes (total WBC), lymphopenia.

10.2.3.2 Cardiovascular: Acute Vascular/Capillary Leak syndrome (CTEP defined section **3.3.1.1**), edema, hypotension, pericardial effusion/pericarditis, PTT, INR (International Normalized Ratio of prothrombin time).

10.2.3.3 Constitutional: fatigue, fever, weight gain.

10.2.3.4 GI: nausea, vomiting, diarrhea.

10.2.3.5 Hepatic: AST, SGOT, ALT, SGPT, GGT, Albumin, serum-low (hypoalbuminemia), alkaline phosphatase.

10.2.3.6 Metabolic/Laboratory: bicarbonate, CPK, Calcium, serum-low (hypocalcemia), Potassium, serum-low (hypokalemia), Magnesium, serum-low (hypomagnesemia), Sodium, serum-low (hyponatremia), Phosphate, serum-low (hypophosphatemia).

10.2.3.7 Musculoskeletal: muscle weakness.

10.2.3.8 Pain: myalgia.

10.2.3.9 Renal: creatinine, proteinuria.

10.3 Record Keeping

10.3.1 Complete records must be maintained on each patient; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, a copy of the signed consent, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. The primary source documentation will assure the following: 1. on-study information, including patient eligibility data and patient history, 2. flow sheets, 3. specialty forms for pathology, radiation, or surgery, and 4. off-study summary sheets, including a final assessment by the treating physician.

10.3.2 An electronic research record including the following items will be kept on the study database: 1. On/off study dates, 2. response and progression dates, 3. drug administration with dose and cycle, 4. toxicity with grade and attribution, 5. concomitant medications.

10.3.3 All patients must have given an informed consent and an on-study confirmation of eligibility form will be filled out before entering on the study.

10.3.4 The data will be submitted electronically from the study database to CTEP.

11 REGULATORY AND OPERATIONAL CONSIDERATIONS

11.1 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).

11.2 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 a 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.

11.3 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.

11.4 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/each 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 stored at the 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 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.

12 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

12.1 IMMUNOTOXIN PHARMACEUTICAL SECTION

LMB-2 (NSC 676422)

Other Names: Anti-TAC (Fv) PE 38.

Classification: Recombinant immunotoxin.

Description: LMB-2, a 63-kD single-chain recombinant immunotoxin, is comprised of variable regions of the light and heavy chains (Fv) of a murine monoclonal antibody (anti-TAC) against the 55-kD subunit of the low affinity interleukin-2 receptor (IL-2R) fused to a truncated derivative of *Pseudomonas exotoxin*, (PE 38).

Mode of Action: The human IL-2R (also known as TAC antigen and CD25) plays an important role in lymphocyte differentiation and immune response regulation. It is overexpressed on various types of malignant cells and lymphocytes mediating autoimmune disease, making IL-2R a potential cancer therapy target. Anti-TAC is a murine monoclonal antibody that binds to the IL-2R alpha with high affinity blocking the interaction of IL-2 with IL-2R. *Pseudomonas exotoxin* (PE) 38 is a truncated portion of a protein secreted by *P. aeruginosa* that lacks the native cell-binding site and kills mammalian cells by catalyzing irreversible ADP-ribosylation and inactivating elongation factor 2, halting protein synthesis. LMB-2 selectively binds to cells bearing IL-2R and is internalized to release PE 38, causing cell destruction.

How Supplied: LMB-2 is available as a sterile frozen solution in phosphate buffered saline and may be vialed and labeled at different concentrations (i.e. 420 mcg/mL, 436 mcg/mL, 484 mcg/mL, 534 mcg/mL, etc.). The pH is approximately 7.4.

Please note: The concentration of LMB-2 may vary from lot to lot. Please check the label for the correct concentration prior to the preparation of each dose.

Preparation: Thawing instructions: Warm vials in the hand for 10 to 20 seconds before placing them in a water bath to thaw. Place vials in a cup of room temperature (15-30ºC) sterile water for injection, USP such that when the vial is upright, the water level will be at the neck of the vial. Visually inspect the vials after thawing. Do not use if solution appears turbid. Do not shake; proteins can foam and may denature.

LMB-2 should only be diluted in 0.2% human serum albumin (HSA) in 0.9% sodium chloride. **Please note:** Particulate matter was found in vials from lots 103037 and 103038 during the 60-month stability testing. The lots met all other release specifications, including composition and potency. Tests were conducted with a Millex GV 25 mm (0.2 micron) filter to remove the particulates. Post filtration studies demonstrated minimal loss of potency. **LMB-2 undiluted solution must be filtered with a 0.2 micron low protein binding Millex GV filter prior to adding to the 0.2% HSA in 0.9% sodium chloride.**

IV infusion: The required amount of LMB-2 (436 mcg/mL, the undiluted vial) will be diluted with 0.2% HSA in 0.9% sodium chloride to a total volume of 50 mL in an empty Partial Additive Container (PAB®). Filter LMB-2 with a 0.2 micron low protein binding Millex GV filter prior to adding to the 0.2% HSA in 0.9% sodium chloride. Agitate gently to disperse.

A PAB® container is a standard, commonly-used parenteral product container that is composed of

an ethylene and propylene co-polymer without plasticizer. It is an empty sterile bag to which pharmacy personnel add the various components specified by the protocol to a specific prescribed volume. It is preferred over other plastic containers because it is manufactured without polyvinylchloride (PVC) and plasticizers such as di-(2-ethylhexyl) phthalate (DEHP) with which some chemotherapy agents interact.

Storage: Intact vials should be stored in the freezer at -70ºC or below. The intravenous admixture should be stored in the refrigerator (2-8ºC). Thawed vials should not be refrozen.

Stability: Intact vials of LMB-2 are stable for at least 5 years when stored at -70ºC. Once thawed, intact vials are stable for 24 hours when stored in the refrigerator (2-8ºC) and for 4 hours when stored at room temperature (15-30ºC). LMB-2 is stable for 25 hours at 2-8ºC once further diluted in 0.2% HSA in 0.9% sodium chloride. Vials cannot be refrozen.

Route(s) of Administration: Intravenous (IV).

Method of Administration: Treatment doses should be infused intravenously over 30 minutes.

Patient Care Implications: If necessary, supportive care for vascular/capillary leak syndrome should be instituted and may include fluid and electrolyte management, diuresis, albumin, glucocorticoids, and cardiovascular support. Other toxicities should be managed clinically.

Anti-emetics or hematologic growth factors are not expected to be required but are permitted if indicated. Patients who develop nausea may be treated with a serotonin 5-HT3 receptor antagonist for at least 24 hours after their last episode of nausea. Other antiemetics, such as prochlorperazine, metoclopramide, or lorazepam may be used in addition if necessary.

Patients who develop myalgias may be given acetaminophen 650 mg to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2.

It may then be given as needed. Patients may receive NSAIDs or narcotics if acetaminophen is inadequate. Patients who develop temperatures >38.0ºC may receive scheduled acetaminophen 650 mg to 1000 mg every 6 hours until 24 hours after completing the last dose of LMB-2. It may then be given as needed.

Emergency medications should be available in the treatment unit in the event of an anaphylactic reaction. Allergic reactions should be treated acutely with antihistamines, and if needed, with glucocorticoids, fluids, and/or epinephrine.

12.1.1 Toxicity of LMB-2

12.1.1.1 Preclinical studies. In a GLP toxicology study, 4 Cynomolgus monkeys received 20 mcg/Kg·dose days 1, 3 and 5 with no significant toxicity. Another four monkeys were then given 300 mcg/Kg·dose days 1, 3 and 5 and experienced dose-limiting toxicity with anorexia and 2 to 4-fold transaminase elevations. The LD_{10} and LD_{50} in mice were 200 and 257 mcg/Kg·dose every other day for 3 doses. The cause of death was liver damage.

12.1.1.2 Phase I grade III-IV toxicities. Adverse events were reported in relationship to treatment cycle. Grade III-IV toxicities included reversible transaminase elevation, fever, CK elevation, cardiomyopathy, thrombocytopenia, allergic reaction, and diarrhea.

12.1.1.3 The most common grade I-II toxicities were transaminase elevation, fever, hypoalbuminemia, and fatigue. Other grade I-II toxicities included vascular leak

syndrome, weight gain, hypotension, nausea, pericardial effusion, allergy, proteinuria, and increased creatinine.

12.1.2 Agent Ordering and Agent Accountability

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

12.1.2.2 LMB-2, NSC 676422 may be requested by completing a Clinical Drug Request (NIH-986) and mailing it to the Drug Management and Authorization Section, PMB, DCTD, NCI, 9000 Rockville Pike, EPN Room 7149, Bethesda, MD 20892-7422 or faxing it to (301) 480-4612. For questions call (301) 496-5725.

12.1.2.3 Agent Inventory Records - The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record (DAR) Form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

12.2 CHEMOTHERAPY PHARMACEUTICAL SECTION

12.2.1 **Cyclophosphamide** (CTX, Cytoxan, NSC-26271)

12.2.1.1 Availability – Cyclophosphamide will be obtained commercially and is supplied as a 2 gram lyophilized powder.

12.2.1.2 Storage and Stability - The vials are stored at room temperature and Reconstituted vials and diluted solutions are stable under refrigeration for 14 days.

12.2.1.3 Preparation - will be reconstituted with sterile water for injection to yield a final concentration of 20 mg/ml as described in the package insert.

12.2.1.4 Route of Administration - The cyclophosphamide used in this regimen will be mixed in 100 ml 0.9% sodium chloride injection and given as an IVPB over 60 minutes. Patients will receive hydration prior to and after administration.

12.2.1.5 Toxicity:

12.2.1.5.1 Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines (e.g., lorazepam).

12.2.1.5.2 Water retention – cyclophosphamide metabolite may cause renal tubular injury which may mimic the clinical picture of inappropriate antidiuretic hormone secretion, usually manifested 4-8 hrs after I.V. administration, necessitating frequent accurate (q 1-2 hrs) assessment of intake, urine output and urine specific gravity. Effect can be counteracted by furosemide. Fluid restriction is not feasible during administration of high dose cyclophosphamide.

12.2.1.5.3 Cardiomyopathy - cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed in patients receiving a higher dose of cyclophosphamide (1200 mg/m^2) used in transplant protocols.

12.2.1.5.4 Hemorrhagic cystitis - this is a serious, potentially life-threatening complication related to the interaction of cyclophosphamide metabolites and the bladder epithelium. Although subclinical hematuria is not uncommon at this dose level, clinically significant hematuria or serious hemorrhage can usually be avoided by maintaining a high urine volume and frequent voidings. The patient will receive 1 liter 0.9% sodium chloride prior to each dose of cyclophosphamide.

12.2.1.5.5 Careful monitoring of serum and urine electrolytes is mandated. Furosemide may be required to insure this diuresis. Bladder irrigation (Murphy's drip) may be used for control of significant hematuria, where saline is infused into the bladder to prevent clot formation.

12.2.1.5.6 Sterility

12.2.1.5.7 Myelosuppression

12.2.1.5.8 Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

12.2.2 **Fludarabine** (Fludara, Berlex Laboratories)

12.2.2.1 Availability - Fludarabine monophosphate is commercially available as Fludarabine, and is supplied as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludara is stored at room temperature.

12.2.2.2 Storage and Stability - Reconstituted Fludarabine is chemically and physically stable for 24 hours at room temperature, or for 48 hours if refrigerated. Because reconstituted Fludarabine contains no antimicrobial preservative, care must be taken to assure the sterility of the prepared solution; for this reason, reconstituted FLUDARA IV should be used or discarded within 8 hours.

12.2.2.3 Preparation - Fludarabine should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7-8.5. Fludarabine will be mixed in 100 ml of 0.9% NaCl.

12.2.2.4 Administration - Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine will be infused I.V. over 30 minutes.

12.2.2.5 Toxicity - Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia, autoimmune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine

monophosphate. Such toxicity has only rarely been demonstrated at the 25-30 mg/m2/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agents.

12.2.3 **Filgrastim** (granulocyte colony-stimulating factor, G-CSF, filgrastim, Neupogen)

12.2.3.1 Availability – Filgrastim will be obtained commercially and is supplied in 300 μg/1 ml vials.

12.2.3.2 Storage and Stability – filgrastim should be refrigerated and not allowed to freeze. The product bears the expiration date. It is generally stable for at least 10 months when refrigerated.

12.2.3.3 Preparation – The appropriate dose is drawn up into a syringe.

12.2.3.4 Administration – filgrastim will be given as a daily subcutaneous injection. Prescribers will be permitted to round down to doses within 10% of the patient's calculated dose to use the drug cost-effectively.

12.2.3.5 Toxicities - The side effects of filgrastim are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) and elevated blood chemistry levels.

12.2.3.6 Relevance to protocol – Rather than wait until patients have fever and neutropenia, since ATL patients are quite immunosupressed even without neutropenia, filgrastim will be given prophylactically after grade III or higher neutropenia is observed. Filgrastim will be administered at 300 mcg qd from d5 to 1 day prior to the next cycle of FC or until the ANC > 5000.

12.3 PREMEDICATIONS (ABBREVIATED PHARMACEUTICAL SECTION)

These agents will be provided by the Clinical Center Pharmacy and will be given orally. Please refer to the package inserts for complete pharmaceutical information on these products.

12.3.1 **Acetaminophen (Tylenol)**: Side effects are unlikely. Regular use of acetaminophen can cause liver damage especially at high doses (>4000 mg/day or >12 regular strength tablets per day). To minimize this possibility patients should not take over-the-counter products containing acetaminophen during the time periods they are taking scheduled acetaminophen doses on this study.

12.3.2 **Ranitidine (Zantac)**: Side effects include tiredness, dizziness, headache, and diarrhea.

12.3.3 **Hydroxyzine (Atarax)**: Side effects include sleepiness, dizziness, restlessness, and irritability.

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14 APPENDICES

14.1 APPENDIX A: SCHEMA & CALENDAR

	Pre[13]	Pre-Cycle [6]	d1	d2	d3	d5	d7	d8-11	F/U[14]
FC administered cycles 1-7			X	X	X				
LMB-2 administered cycles 2-7					X	X	X		
Immunohistochemistry	X[1]								
Hepatitis B & C[9], HIV, PFTs, CMV/EBV PCR	X[2]								
Lipid panel, fibrinogen, thrombin time, TSH, Free T3, Free T4, T3, T4, 24 hour urine for creatinine clearance, protein, and UPEP, Exercise Stress Test (ETT)	X[10]								
Pregnancy test	X[3]								
Physical exam, & if relevant, dermatology consult, clinical photography	X[4]	X							X
Flow cytometry	X[4]	X[7]						X	X
Imaging with CT chest, abdomen, pelvis, neck, MRI, U/S, or PET/CT (PI discretion)	X[4]	X							X
CBC + diff, Chemistries[11], urinalysis	X[5]	X[7]	X		X	X	X	X	X
PT, PTT, IgA, IgG, IgM, GGT, haptoglobin, amylase, lipase	X[5]	X						X	X
sCD25 (& other tumor markers),	X[5]	X[7]			X			X	X
Neutralizing antibodies	X[5]	X[7]						X	X
Pharmacokinetic Studies (per section **3.4**)					X	X	X		
CRP, beta-2-microglobulin	X[5]	X			X			X	X
Echocardiogram	X[2]	X							
Chest X-ray		X[12]						X	
Electrocardiogram (EKG)	X[1]	X[7]				X[8]	X		

[1]Any time before enrollment
[2]Within 2 months before enrollment
[3]Within 1 week before enrollment
[4]Within 2 weeks before enrollment
[5]Within 3 days before enrollment
[6] Begin cycle 2 on day 14-43 and cycles 3-7 on day 21-50 of the previous cycle. Do precycle tests 0-3 days before the cycle begins. Day 1 is considered precycle.
[7] Also draw at study exit or completion
[8] EKG to be performed 10-30 min after end of infusion day 5 and before dosing day 7
[9]HBsAg and core Ab
[10]Within 28 days before enrollment
[11] Chemistries (Chem-20): Albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CK, chloride, CO2, creatinine, direct bilirubin, glucose, potassium, LDH, magnesium, sodium, phosphorus, total protein, uric acid
[12]Chest X-ray pre-cycle if no chest CT has been obtained
[13] EKG, CRP and beta 2 microglobulin, PT/PTT, IgA, haptoglobin and urinalysis are baseline tests, all others are used to determine research eligibility
[14] Follow-up tests performed at up to 6-month intervals, at discretion of PI

14.2 APPENDIX B: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Descriptions
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.