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Principal Investigator: Robert Kreitman NCI LMB 301.496.6947 kreitmar@mail.nih.gov

(NIH Employee Name, Institute/Branch, Telephone and e-mail)

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SIGNATURES

Principal Investigator (*):
Robert Kreitman - applied signature on 05/07/2015 4:13 PM EDT

Accountable Investigator:
PI is the Accountable Investigator

Branch Chief/CC Department Head (**):
Ira Pastan - applied signature on 05/07/2015 3:39 PM EDT

Medical Advisory Investigator (if applicable):
N/A

Lead Associate Investigator signature:
N/A

Referral Contact signatures:
N/A

Associate Investigators signatures:
Julie Feurtado - applied signature on 05/08/2015 3:53 PM EDT

For Institute/Center Scientific Review Committee:
N/A

Other IC Clinical Director signatures:
N/A

APPROVALS

IRB Chair:
Michael Hamilton - applied signature on 05/21/2015 11:16 AM EDT

Clinical Director:
William Dahut - applied signature on 05/14/2015 6:16 PM EDT

CONCURRENCE

OPS Protocol Specialist:

Tiffany Johnson	AM O	5/22/2015
Signature	Print Name	Date

* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

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

Abbreviated title: LMB-2 and FC in ATL

Principal Investigator and Affiliation:
Robert J. Kreitman, M.D., Laboratory of Molecular Biology (LMB), Centers for Cancer Research (CCR), National Cancer Institute (NCI), NIH (9000 Rockville Pike, Bethesda, MD 20892), room 37/5124b, Phone 301-496-6947, email kreitmar@mail.nih.gov

Associate Investigators:

Wyndham Wilson, M.D., Ph.D., Lymphoid Malignancies Branch (LYMB), CCR, NCI, NIH, room 10-CRC/4-3132, Phone 301-435-2415, email wilsonw@mail.nih.gov

Kevin Conlon, M.D. LYMB, 10-CRC/4-5322, Phone 301.496.7417, conlonkc@mail.nih.gov

Ira Pastan*, M.D., LMB, 37/5106, Phone 301-496-4797, email pastani@mail.nih.gov

Thomas A. Waldmann*, LYMB, 10/4N115, Phone 301-496-6663, waldmannt@mail.nih.gov

Maryalice Stetler-Stevenson*, M.D., Ph.D., Laboratory of Pathology (LP), CCR, NCI, NIH, room 10/B1/56-58, phone 301-402-1424, email stetler@mail.nih.gov

Constance M. Yuan*, M.D., Ph.D., LP, 10/2N108, 301-496-4709, yuanc@mail.nih.gov

Seth Steinberg*, Ph.D., Biostatistics and Data Management section, CCR, NCI, NIH, 9609 Medical Center Drive, 2W334, phone 240-276-5563, email steinbes@mail.nih.gov

Lesley Mathews, C.R.N.P., LMB, CCR, NCI, NIH, room 10/12N214, phone 240-586-0109, email lesley.mathews2@nih.gov

Laura Wisch*, R.N., OCD, CCR, NCI, room 10/12N214, phone 301-594-1827, email wischl@mail.nih.gov

Theresa Yu*, R.N., OCD, CCR, NCI, room 10/12N214, phone 301-496-9458, email theresa.yu@nih.gov

Julie C. Feurtado, RN, OCD, CCR, NCI, room 10/12N214, phone 301-402-5633, email julie.feurtado@nih.gov

Referral Contact:
Laura Wisch*, R.N., OCD, CCR, NCI, room 10/12N214, phone 301-594-1827, email wischl@mail.nih.gov

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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.

Objectives:

- 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.
- 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.

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 patients, which includes 4 replacements.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary

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

- 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.

1.2.5 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.

1.2.6 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.

1.2.7 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).

1.2.8 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.

1.2.9 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).

1.2.10 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.

1.2.11 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.

1.2.12 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.

1.2.13 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.

1.2.14 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.

1.2.15 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.

1.2.16 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:

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

1.2.16.2 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.

1.2.16.3 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).

1.2.16.4 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.

1.2.16.5 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).

1.2.16.6 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.

1.2.16.7 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.

1.2.16.8 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).

1.2.16.9 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).

1.2.16.10 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).

1.2.16.11 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).

1.2.16.12 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).

1.2.16.13 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).

1.2.16.14 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).

1.2.17 Immunogenicity of Mabs and immunotoxins

1.2.17.1 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).

1.2.17.2 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).

1.2.17.3 Immunogenicity of LMB-2. As mentioned in section **1.2.10**, 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.

1.2.18 Reducing humoral immunity with chemotherapy and other agents

1.2.18.1 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.

1.2.18.2 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.

1.2.18.3 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.

1.2.18.4 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.

1.2.18.5 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.

1.2.18.6 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 **1.2.18**), 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 **1.2.18.6**), 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 1.2.16.6) (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 **1.2.17.3**, 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 **2.2.5**).

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.

2.2 RESEARCH ELIGIBILITY EVALUATION

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

2.2.2 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.

2.2.3 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).

2.2.4 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).

2.2.5 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 studyparticipation until 1 month after the last cycle of LMB-2.

2.2.6 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 PATIENT REGISTRATION AND TREATMENT RANDOMIZATION

2.3.1 Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of anyinvestigational agents. Verification of Registration will be forwarded electronically via e-mail. Please note, it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient's information. A recorder is available during non-working hours.

2.3.2 Off-Study Procedure: Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

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 Optional studies (initial and follow-up testing 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, and then with post-treatment follow-up.

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

3.6 CONCURRENT THERAPIES

3.6.1 Listed in **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 OFF TREATMENT CRITERIA: (NO TREATMENT BUT DATA CAN BE OBTAINED)

3.8.1 Progressive disease unless it occurs during a dosing delay for toxicity as specified by protocol section **3.3.3.2**.

3.8.2 > 75% neutralization of 1000 ng/ml of LMB-2.

3.8.3 Grade III Allergic Reaction and grade II urticaria despite premedication.

3.8.4 More than 2 dose reductions of LMB -2.

3.8.5 Grade IV DLT other than ALT, AST, GGT, CPK.

3.8.6 Intercurrent illness or medical circumstances.

3.8.7 General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

3.9 OFF STUDY CRITERIA

3.9.1 Patient decides to withdraw from the study

3.9.2 Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

3.9.3 Data can be collected for 30 days after the last administration of the study drug.

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.

3.10.1 SUPPORTIVE CARE

3.10.1.1 Allergic reaction

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

3.10.1.2 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.

3.10.1.3 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.

3.10.1.4 Vascular/Capillary leak syndrome

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

3.10.1.5 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.

3.10.1.6 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.

3.10.1.7 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.

3.10.1.8 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 be used.

3.10.1.9 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.

3.10.1.10 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.

3.11 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' biopsychosocial well-being.

4 DATA COLLECTION AND EVALUATION

4.1 DATA COLLECTION

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.

4.1.1 Informed consent

The original signed consent goes to Medical Records; copy placed in research record.

4.2 RESPONSE CRITERIA

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

4.2.2 **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.

4.2.3 **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.

4.2.4 **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.

4.2.5 **Partial response (PR)**. Reduction by $\geq$ 50% of leukemia cell count or $\geq$ 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.

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

4.2.7 **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.

4.2.8 **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.

4.3 TOXICITY CRITERIA

CTCAE term (AE description) and grade: 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).

4.4 STATISTICAL CONSIDERATIONS

4.4.1 **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.

4.4.2 **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. If 2 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.

4.4.3 **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%.

4.4.4 **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.

4.4.5 **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.

4.4.6 **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.

4.4.7 **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.

4.4.8 **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.

4.5 MULTI-INSTITUTIONAL GUIDELINES

This is a single institution trial.

4.6 DATA AND SAFETY MONITORING PLAN

4.6.1 The research nurse will ensure that data, reporting, and adverse events will be reviewed at least every other week. Team meetings are held every 1-2 weeks. Unexpected events will be monitored for trends, which if found may lead to protocol amendment or

suspension of accrual. Amendments to the protocols and consents will be made to protect the patients and answer important scientific questions that arise.

4.6.2 Intramural quality assurance monitors will be monitoring the protocols yearly. NCI C3D will be the database used to report data to CTEP. The data is closely monitored by data management contractors from Harris.

4.6.3 All serious adverse events must be reported immediately by telephone to the Principal Investigator, Dr. Robert Kreitman (301-496-6947). Call 301-496-1211 after hours.

4.6.4 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.

4.6.5 Protocol changes must be in the form of a written amendment. Protocol amendments and necessary revisions to the informed consent form must be submitted by the Investigator to the local IRB and such amendments will only be implemented after written approval of the requisite IRB.

5 HUMAN SUBJECTS PROTECTIONS

5.1 RATIONALE FOR SUBJECT SELECTION

5.1.1 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.

5.1.2 **Inclusion of Women and Minorities.** Both men and women and members of all races and ethnic groups are eligible for this trial.

5.2 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.

5.3 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.

5.4 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.

5.5 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.

5.6 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.

5.7 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, and the potential benefits will be carefully explained to the patient or the patient's advocate. This process will include a general description of the disease process, as well as a description of the patient's expected clinical course. Alternative therapies will be carefully explained, and outlined in the consent document. The patient will be asked to read the consent at his/her convenience and will be encouraged to ask questions. Enrollment on this study will only occur if the patient meets all eligibility criteria, is judged by the Investigator to potentially benefit from the therapy, is able and willing to provide full consent, and has signed the consent document. Moreover, any experimental invasive procedure will require a separate consent form (standard procedure consent form). For pre-study screening, investigators will obtain consent for submission of sera and tumor according to the policies of the IRB.

5.8 STORING SPECIMENS

5.8.1 Description of data/specimens: 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 maybe tested with LMB-2 and related drugs to determine if the malignant cells can be killed outside the body. Requires 1-3 tablespoons.

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

5.8.2 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 to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Any freezer problems, lost samples or other problems associated with samples to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

5.8.3 Confidentiality: Patient names or identifiers will not be used in publications resulting from testing of patient samples. Samples shipped to locations other than Frederick will have patients identifiers removed. Other than described above, no germline testing will be done which may impact disease risk in the patient's relatives.

6 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING

6.1 DEFINITIONS

6.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per section **6.3**.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.
- 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.
- Calcium values corrected for albumin will be used to report hypocalcemia.

6.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

6.1.3 Unexpected adverse reaction

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

6.1.4 Serious

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

6.1.5 Serious Adverse Event

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

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

6.1.6 Disability

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

6.1.7 Life-threatening adverse drug experience

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

6.1.8 Protocol Deviation (NIH Definition)

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

6.1.9 Non-compliance (NIH Definition)

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

6.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

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

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

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

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

6.2 ADVERSE DRUG REACTIONS

6.2.1 **Routine reporting**: All observed or volunteered adverse events, regardless of treatment group or suspected causal relationship to study drug, will be recorded in the NCI electronic case report form (via NCI C3D). Adverse events must be reported in a consistent manner for all patients on study, based on the following rules: Adverse events will be identified and graded using the NCI CTCAE version 3 until December 31, 2010. CTCAE version 4.0 will be utilized beginning January 1, 2011. Next, it will be assessed if the adverse event is related to the medical treatment (attribution). If so, it will be determined whether the adverse event is expected or unexpected (see Investigator's Brochure). Patients should be followed for 30 days after the last dose for adverse events, and thereafter for adverse events at least possibly related to study drug. If, in the judgment of the PI, the adverse event is not constant but fluctuates (or stutters) or changes grade during a period of time, it may be reported as one event with the grade being the maximum grade reached, and the resolved date being the date it returns to baseline grade. Clinical judgment of the PI must be used to assess the baseline grade of adverse events.

The laboratory value immediately before beginning drug is usually used to determine grades of baseline laboratory AEs, but laboratory values from the prior 90 days may be used if clinically relevant. All calcium values will be corrected for albumin. To determine the corrected calcium in mmol/L, subtract the albumin in g/dL from 4.0, multiply the result by 0.2, then add the product to the measured calcium in mmol/L.

6.3 NCI-IRB REPORTING

6.3.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

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

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

6.3.2 NCI-IRB Requirements for PI Reporting at Continuing Review

Below is an example of the table used for reporting:

System Organ Class	CTCAE Term	Grade	# of Events since last CR	Total # of Events	Attribution to Research	Serious?	Unexpected?

The protocol PI will report to the NCI-IRB:

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

- All Serious Events regardless of attribution.

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

6.3.3 NCI-IRB Reporting of IND Safety Reports

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

6.3.4 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.

6.3.5 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.

6.4 EXPECTED ADVERSE EVENTS

6.4.1 Grade 4: none

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

6.4.3 Grade 2:

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

6.4.3.2 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).

6.4.3.3 Constitutional: fatigue, fever, weight gain.

6.4.3.4 GI: nausea, vomiting, diarrhea.

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

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

6.4.3.7 Musculoskeletal: muscle weakness.

6.4.3.8 Pain: myalgia.

6.4.3.9 Renal: creatinine, proteinuria.

6.5 RECORD KEEPING

6.5.1 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.

6.5.2 An electronic research record including the following items will be kept on NCI C3D: 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.

6.5.3 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.

6.5.4 The data will be submitted electronically from NCI C3D to CTEP.

6.6 DATA AND SAFETY MONITORING PLAN

6.6.1 All serious adverse events must be reported immediately by telephone to the Principal Investigator, Dr. Robert Kreitman (301-496-6947). Call 301-496-1211 after hours.

6.6.2 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.

7 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

7.1 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□ 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.

7.1.1 Toxicity of LMB-2

7.1.1.1 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.

7.1.1.2 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.

7.1.1.3 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.

7.1.2 Agent Ordering and Agent Accountability

7.1.2.1 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.

7.1.2.2 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.

7.1.2.3 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.)

7.2 CHEMOTHERAPY PHARMACEUTICAL SECTION

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

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

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

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

7.2.1.4 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.

7.2.1.5 Toxicity:

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

7.2.1.5.2 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.

7.2.1.5.3 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.

7.2.1.5.4 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.

7.2.1.5.5 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.

7.2.1.5.6 Sterility

7.2.1.5.7 Myelosuppression

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

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

7.2.2.1 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.

7.2.2.2 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.

7.2.2.3 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.

7.2.2.4 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.

7.2.2.5 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.

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

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

7.2.3.2 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.

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

7.2.3.4 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.

7.2.3.5 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.

7.2.3.6 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.

7.3 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.

7.3.1 **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.

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

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

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9 APPENDICES

9.1 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

9.2 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.

INSTITUTE: National Cancer Institute

STUDY NUMBER: 09-C-0025 PRINCIPAL INVESTIGATOR: Robert J. Kreitman, M.D.

STUDY TITLE: Phase II Trial of LMB-2, Fludarabine and Cyclophosphamide for Adult T-Cell Leukemia

Continuing Review Approved by the IRB on 12/15/14
Amendment Approved by the IRB on 05/21/15 (O) Date Posted to Web: 05/23/15

Eligibility Screening

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Description of Research Study

This consent form is to allow drawing blood and sending samples to determine your eligibility for our study involving a recombinant immunotoxin for the treatment of cancer. The recombinant immunotoxin is a protein containing a toxin part and an antibody part. The antibody part binds to a surface protein (also called antigen) on the surface of the cancer cell and then the toxin goes inside the cell and kills it. In this study the recombinant toxin is called LMB-2 and the antigen it binds to, CD25, is often present on the malignant cells. To determine your eligibility for LMB-2, we would first need to test your tumor or other tissue for the presence of CD25 on the surface of your cancer cells. You will be informed if CD25 is found and if several other requirements are met, you may be eligible for our recombinant immunotoxin study. Whether or not you are eligible for our study, we may obtain follow-up data on your outcome from you or your physician. This includes, if they occur at all, the date of tumor recurrence, tumor progression, and possibly death. However, this consent does not permit any additional studies that would test for genes (i.e. tendency for diseases). Other tests to determine if you are eligible may take several weeks and will most likely be done as an outpatient. These tests may include standard blood and urine tests, an electrocardiogram test of your heart, a chest X-ray, an echocardiogram, which is an ultrasound of the heart, computerized tomography (CT or CAT) scans, X-rays, nuclear medicine studies, and a bone marrow biopsy.

Tests needed to determine whether you are eligible for this trial:

- Flow cytometry of the blood. Requires about 1/2 tablespoon.
- Neutralizing antibodies: Antibodies a patient might make to certain protein drugs which block their effect against cancer cells. You may or may not consider receiving these protein drugs in the future. Requires about 1 teaspoon.

Alternative Approaches or Treatments

You may choose not to be tested for CD25 or to have any other studies done.

Risks or Discomforts of Participation

The risk involves the withdrawal of between a few teaspoons and a half-cup of blood and the potential for bruising or infection that occurs with any blood draw. Your tumor tissue may be obtained from prior surgeries or from a biopsy that you might elect to have for purposes of determining if you are eligible for this study. Any biopsy or other procedure would be done only

if needed and only after you sign an additional informed consent related to the specific procedure.

Potential Benefits of Participation

There may be no direct benefit from allowing us to test your blood or other tissue for CD25 or other factors. However, this testing may make you eligible for our recombinant immunotoxin protocol. If you become eligible for our treatment study you would need to give additional informed consent regarding the risks of the treatment.

Consent for Participation

Upon completion of this study, you may be given the option of participating in additional research protocols if such protocols exist. If they do not, you will be returned to the care of your referring physician. It is important to stress that participation in this protocol does not constitute a promise of long-term medical care here at the NIH Clinical Center. If there is no research study that is suitable for you and your state of disease, you will be returned to the care of your referring doctor or institution, or to alternative sources of care closer to home. It is conceivable that participation in this study may make you ineligible to participate in certain other research protocols. You may decide now not to participate in this protocol, or you may choose at any time to withdraw from the protocol.

Optional Studies (not required)

We would like to keep some of the specimens and data that are collected for future research. These specimens and data will not be identified by name when sent outside the NIH or stored, only by number. The use of your specimens and data will be for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you decide now that your specimens and data can be kept for research, you can change your mind at any time. Just contact us and let us know that you do not want us to use your specimens and/or data. Then any specimens that remain will be destroyed and your data will not be used for future research.

Please read each sentence below and think about your choice. After reading each sentence, circle and initial the answer that is right for you. No matter what you decide to do, it will not affect your care.

1. My specimens and data may be kept for use in research to learn about, prevent, or treat cancer or other health problems.

Yes No Initials_____

2. Someone may contact me in the future to ask permission to use my specimens and/or data in new research not included in this consent.

Yes No Initials_____

Optional Studies (not required to determine if you are eligible)

- Soluble CD22, CD25, CD30, 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 may also be measured in your tumors if they need to be removed as part of your medical care, or if tumors can be easily and safely removed like with a skin biopsy.
- 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. HLA is the human leukocyte antigens, a complex of proteins on your white blood cells which allow your body to determine whether the cell is yours or not. 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. PAX-gene tubes contain a special liquid that keeps RNA in the blood stable, and it mixes with your blood only after it is drawn. Requires about 1/2 teaspoon.
- DNA samples to look for abnormalities which might make a patient more susceptible to toxicity. The genes to look at would include those that trigger cells to die, and those that help make hormones which cause inflammation. Requires about 1/2 teaspoon.

- Assays to identify genes in your DNA which would have significant risk to you, including how likely you or your children might be to get cancer, will not be done.
- 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. In flow cytometry, your blood after being drawn goes into a tiny tube where lasers determine whether the tumor markers are present if so how much. 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.

Disclosure of potential conflict of interest:

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The National Institutes of Health and the research team for this study have developed a drug, being used in this study. This means it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development of LMB-2.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Robert J. Kreitman, M.D.; Building 37, Room 5124b, Telephone: 301-496-6947. Other researchers you may call are: Theresa Yu, R.N., Telephone: 301-496-9458. You may also call the Clinical Center Patient Representative at 301-496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/ Legal Representative Date

Print Name

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian Date

Print Name

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian Date Print Name

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM DECEMBER 15, 2014 THROUGH JUNE 14, 2015.

Signature of Investigator Date

Print Name

Signature of Witness Date

Print Name

INSTITUTE: National Cancer Institute

STUDY NUMBER: 09-C-0025 PRINCIPAL INVESTIGATOR: Robert J. Kreitman, M.D.

STUDY TITLE: Phase II Trial of LMB-2, Fludarabine and Cyclophosphamide for Adult T-Cell Leukemia

Continuing Review Approved by the IRB on 12/15/14
Amendment Approved by the IRB on 05/21/15 (O) Date Posted to Web: 05/23/15

Standard

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Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Description of Research Study

This is a clinical trial for the treatment of adult T-Cell leukemia with an experimental drug called LMB-2 after the combination of the chemotherapy drugs fludarabine and cyclophosphamide (FC). The 2 drugs in addition to LMB-2 are abbreviated FC. You will be treated with FC on days 1, 2, and day 3 for each of 7 cycles. The beginning of the 2nd cycle is about 2 weeks after first, and after that the cycles are about 3 weeks apart. Starting with the 2nd cycle, on the last day of FC (day 3), you will be treated with LMB-2 every other day for 3 doses. After this 2nd cycle, you can be retreated with FC/LMB-2 a maximum of 5 more times provided certain requirements are met. LMB-2 is a recombinant immunotoxin that has been shown to kill leukemia and lymphoma cancer cells that have a protein on their surface called "CD25". To be eligible for treatment on this study, your malignant cells must have CD25 on their surface. However, the presence of CD25 on your malignant cells does not guarantee that you will be enrolled on the protocol. We plan to include at most 37 patients on this trial.

LMB-2 is an experimental drug called a recombinant immunotoxin. Each LMB-2 molecule is made up of two parts: a protein part that binds or targets a cancer cell and a toxin (a type of poison) part that goes inside and kills the cancer cell. The binding part is part of an antibody that binds to human CD25, a protein on your leukemia or lymphoma cells. The toxin portion of LMB-2 is naturally produced by bacteria. In laboratory experiments using leukemia or lymphoma cells outside the body, LMB-2 kills cells which have CD25 on their surface. LMB-2 also causes a significant decrease in the size of tumors in mice that were given doses similar to those used in the first human trial of LMB-2.

A preliminary study of LMB-2 has been performed at the National Cancer Institute (NCI) in which 39 patients with various leukemias and lymphomas were treated. In that trial, patients with hairy cell leukemia (4 patients), cutaneous T cell lymphoma (1), adult T-cell leukemia/lymphoma (1), Hodgkin's disease (1) and chronic lymphocytic leukemia (1) had reduction in their tumors.

LMB-2 Treatment

LMB-2 will only be given to patients at the NIH Clinical Center. Each cycle of LMB-2 is given by an intravenous (into a vein) infusion every other day for 3 doses. You will receive up to 6 cycles of LMB-2 every 3 weeks unless you develop worsening of disease, serious side effects, or voluntarily withdraw.

A small amount of blood (up to 10 teaspoons) will be drawn before, during, and after treatment. These blood tests allow us to measure how much LMB-2 is in your blood, the effects of LMB-2

on your cancer cells in your blood, and monitor for side effects. We will also do blood tests prior to each cycle and during each cycle to know how your immune system is interacting with LMB-2.

Before each cycle and in follow-up visits you will undergo repeat disease evaluation. This will include a careful physical examination, blood and urine tests, and tumor-measuring scans like computed tomography (CT) scan. If these studies help us understand how your leukemia or lymphoma is reacting to FC chemotherapy and LMB-2, we may ask for your permission to repeat these tests again prior to other cycles.

The infusion of LMB-2 takes 30 minutes. You will also receive a liter (about 8 cups) of fluid through an IV or central venous catheter before and after each dose of LMB-2. A central venous catheter (CVC) is a plastic IV tube that is placed in a large vein that leads to the heart. You may already have a CVC in place. If not, depending on the size of your arm veins, one may need to be placed prior to treatment. A CVC makes treatment on this study easier and less painful by decreasing the need for IVs and needle sticks to draw blood. If a CVC is required or requested, you will be asked to review another consent form and give consent prior to its placement.

You will receive LMB-2 as an inpatient (admitted to the hospital) or as an outpatient (not admitted to the hospital). If the infusions are well tolerated, you may return home after about 1 week (possibly longer if complications occur). After returning home, you will have blood tests done weekly and the results will be faxed to us by your local physician. During the course of this study, you may also require other treatments such as transfusions and antibiotics. Hospitalization may be needed if complications develop. If there is evidence that therapy with LMB-2 is no longer effective, it will be discontinued.

Fludarabine and cyclophosphamide chemotherapy

One goal of the fludarabine and cyclophosphamide (FC) chemotherapy (FC) is to prevent neutralizing antibodies (antibodies your body makes which inactivate LMB-2) from forming once you receive LMB-2. Since LMB-2 contains a bacterial toxin, your body may make antibodies to it which will eliminate the ability of LMB-2 to bind to malignant cells and kill them. Experience in patients suggests but does not prove that FC may decrease the risk that you would make neutralizing antibodies to LMB-2. Another goal for the FC is to help the LMB-2 work better by first making the tumor smaller. Because the FC and LMB-2 are given so close together, it may be impossible to determine whether the LMB-2 is helping as well. However,

during the trial we will be determining whether you respond well and make antibodies to LMB-2, and also other measures of your immunity. FC chemotherapy will be administered intravenously, daily for 3 days (days 1-3). It is possible that FC may harm your immune system without preventing neutralizing antibodies to LMB-2. The FC chemotherapy could cause problems because of a decreased number of neutrophils, which are a type of infection-fighting white blood cell. If you have fever and low neutrophils, you may need to take filgrastim (G-CSF) then and on future cycles where FC is given. G-CSF is given by an injection under the skin starting on day 5 and continuing until the absolute neutrophil count has exceeded 5000, or 1 day prior to the next cycle. The LMB-2 will begin on the 3rd day of the FC chemotherapy, and will be repeated every other day for a total of 3 doses. Blood will be drawn prior to each cycle of LMB-2 to determine if the level of neutralizing antibodies is too high to give additional LMB-2. If it is too high, further LMB-2 will not be given. Because the result may not be known before the next cycle, you may receive one cycle of LMB-2 in the presence of antibodies. If you don't make antibodies and your disease is stable or decreasing with FC and LMB-2, you may continue to receive FC and LMB-2 for up to a total of 6 cycles each beginning 3 weeks apart.

Alternative Approaches or Treatments

You may decide now not to receive treatment in this protocol or you may choose at any point in time to stop the drug and withdraw from the protocol. In either case you would be returned to the care of your referring physician.

Because of the type and extent of your tumor, chemotherapy is felt to be more beneficial than surgery or radiation alone. Alternative approaches that could be used may include:

1. Other forms of treatment:
    a. Additional chemotherapy drugs and chemotherapy drug combinations which might be of benefit for your disease.
    b. Radiation treatment, which sometimes can control tumor growth in local areas such as lymph nodes, spleen and bones. However, this approach will not effectively treat disease that has spread beyond the areas that are irradiated.
    c. Surgery, which can be used to remove tumor that is pressing on important body parts due to fast growth.
2. Other experimental agents that have not been conclusively demonstrated to be effective.
3. Getting no treatment.
4. Getting comfort care, also called palliative care. This type of care helps reduce pain, tiredness, appetite problems, and other problems caused by cancer. It does not treat the

cancer directly, but instead tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.

Risks or Discomforts of Participation

Administration of FC and LMB-2 will be through a central venous catheter (CVC) or a peripheral I.V. The CVC is inserted by experienced staff using local anesthesia. The risks associated with the procedure include pain, bleeding, infection, and development of air in the chest. However, these complications are rare. Air in the chest outside the lung may require temporary placement of a chest tube by a surgeon. The risks of chest tube placement include pain, bleeding, and infection. Other risks of the catheter include infection and clotting of your veins, which could require removal of the catheter for treatment. These risks will be explained to you in more detail at the time of insertion. When a peripheral line is used, there is a small risk of infection, clot or bleeding at the site of the IV line. There is also a risk of some of the drug leaking out, also called extravasating. If that occurs there may be some destruction of skin tissue in a limited area. Patients are urged to alert the study physicians at the first sign of any skin changes, for example redness or tenderness, around the infusion site but also with any discomfort in the involved extremity as well. If there is any evidence of toxicity from leaking, the infusion will be held until a central line can be placed for the infusion of drug. In addition, any toxic effects to the skin will be treated to the fullest extent possible.

LMB-2:
There is limited experience with LMB-2 in humans. In the Phase I trial, a total of 39 patients received 65 cycles of LMB-2. On that trial, all side effects of LMB-2 went away when LMB-2 was stopped. In some cases this required additional medical treatments. Possible side effects of LMB-2 are summarized below:

Possible:

- Bloating, diarrhea
- Anemia which may require blood transfusions
- Pain
- Dizziness, headache
- Swelling of the body
- Fluid in the body which may cause low blood pressure, shortness of breath, swelling of ankles
- Abnormal heartbeat

- Decrease in heart's ability to pump blood during the "active" phase of the heartbeat (systole)
- Diarrhea
- Nausea or the urge to vomit
- Vomiting
- Swelling of the extremities (arms and/or legs)
- Fatigue or tiredness
- Fever
- Abnormal reaction of the body to substances, called allergens, that are contacted through the skin, inhaled into the lungs, swallowed, or injected (allergic reaction)
- Bruising, bleeding
- Increased blood level of a liver enzyme (ALT/SGPT)
- Increased blood level of a liver or bone enzyme (alkaline phosphatase)
- Increased blood level of a liver enzyme (AST/SGOT)
- Increased blood level of enzyme (creatine phosphokinase) from muscle
- Increased blood level of creatinine (a substance normally eliminated by the kidneys into the urine)
- Decreased number of a type of blood cell that help to clot blood (platelet)
- Weight gain, loss of appetite
- Decreased levels of a blood protein called albumin
- Muscle pain
- Blood in the urine
- More protein in the urine than usual, often a sign of kidney disease
- Shortness of breath
- Increase in the number and size of the pores in the capillaries (small blood vessels) which causes leakage of fluid from the blood to the tissue spaces, resulting in dangerously low blood pressure, swelling and multiple organ failure
- Heart failure which may cause shortness of breath, swelling of ankles, and tiredness
- Low blood pressure which may cause feeling faint

A common side effect of immunotoxin drugs similar to LMB-2 is vascular leak syndrome, where fluid leaks out of blood vessels into the skin, lungs, and other organs. The symptoms may include swelling, weight gain, loss of blood pressure, shortness of breath. This can be severe, and although vascular leak syndrome usually gets better, it may require intubation and can be fatal. Patients may have protein leak into the urine. Other side effects associated with immunotoxins include edema (swelling), aches and pains of the muscles, joints, and/or bones, headache, fatigue, dizziness, blurred vision, lowering of normal blood cells including the red

cells (with risk of anemia), white cells (with risk of infection), and platelets (with risk of bleeding), abnormal blood clotting tests and risk of bleeding, muscle damage, diarrhea, constipation, stomach or intestinal ulcers, stomach pain, indigestion, dehydration, kidney damage, abnormal blood salt levels, fluid leak in the lungs with shortness of breath, inflammation of the pancreas gland (the organ involved in diabetes), chills, decreased function of the thyroid gland, and neurologic problems including sleepiness, decreased level of alertness, weakness, painful tingling ("pins and needles"), numbness (decreased feeling), and coma. It is not known whether LMB-2 poses a risk to the developing fetus and breast-fed infants. All patients with childbearing potential, both male and female, must agree to use effective measures of contraception while on study. Patients that are pregnant or breast-feeding will be excluded from this trial because the effect of LMB-2 on a developing fetus or a nursing infant is unknown and potentially harmful. Whether you are a male or female, you should practice contraception for at least one month after receiving the last dose of LMB-2 or chemotherapy on this trial.

A condition known as hemolytic uremic syndrome (HUS) has been seen with related immunotoxin drugs although not with LMB-2. HUS is a potentially fatal problem that can cause fever, anemia (low red blood cell count), thrombocytopenia (low platelet count), bleeding, stroke, and kidney failure. Treatment of severe HUS includes a procedure known as plasma exchange or plasmapheresis, where the liquid portion of the blood (plasma) is removed from the body and replaced with plasma from blood donors using a special machine. Even with treatment, HUS may lead to death or permanent kidney and/or brain damage. Adverse reactions associated with plasmapheresis are rare, and are generally mild. They include pain and bruising at the insertion site of the intravenous line, and a temporary decrease in the platelet count and/or red blood cell count. Fainting episodes related to needle insertion can occur, and skin tingling caused by low calcium levels can rarely occur. Interrupting the plasmapheresis procedure can reverse this latter reaction. During plasmapheresis, at least two nurses will be present, and a blood bank physician will be available in the clinic area where the procedure is performed.

LMB-2 and other similar drugs can cause allergic reactions that may range from mild to severe. Symptoms of allergic reactions may include hives (red rash with bumps, wheals, or welts), and other skin rashes, swelling, itching, fever, chills, low blood pressure, fast heart rate, wheezing, shortness of breath, and rarely, death. In an attempt to decrease the risk of such reactions, you will be given a number of additional medications ("Premedications") before and after each dose of LMB-2.

Cyclophosphamide toxicity

a. Nausea and vomiting - variable; can be improved with standard medications (e.g., lorazepam).

b. Water retention – cyclophosphamide inside the body may turn into a drug which can hurt the kidney. This side effect can be counteracted by a diuretic called furosemide.

c. Cardiomyopathy - cyclophosphamide may cause severe, sometimes fatal, heart damage. Patients may present with heart failure as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed mainly in patients receiving a higher dose of cyclophosphamide (1200 mg/m^2) used in transplant protocols.

d. Hemorrhagic cystitis - this is a serious, potentially life-threatening complication to the bladder. Although unnoticeable blood in the urine is not uncommon at this dose level, clinically significant blood in the urine can usually be avoided by maintaining a high urine volume and frequently urinating. The patient is encouraged to drink plenty of fluids, at least 2 liters per day, while taking FC.

e. Sterility

f. Less common but serious complications include lung damage and new cancers. Less common but temporary toxicities include balding and skin rash.

Fludarabine toxicity

Fludarabine toxicities include bone marrow damage with low blood counts, fever, nausea, vomiting, sores in the mouth, diarrhea, stomach bleeding, low appetite, swelling, skin rashes, muscle aches, headache, nervousness, hearing loss, temporary episodes of fatigue, destruction of red cells and platelets by the body's immune system, abnormal sensations, kidney damage, and lung damage. Severe fatal brain toxicity with loss of vision and progressive deterioration of mental status were seen almost exclusively after very high doses of fludarabine. This toxicity has only rarely been seen at the dosage of fludarabine used for this trial. Very rarely described complications include severe autoimmune disease and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky tumors. The symptoms of tumor lysis syndrome include rapid reduction in tumor size, fever, low blood pressure, abnormal blood chemistry results, and decreased kidney function. Unusual infections seen in immunosuppressed people (protozoan, viral, fungal, and bacterial) have been observed after-fludarabine, especially in heavily pre-treated patients, and in patients receiving fludarabine combined with other agents.

Patients with lymphoma and leukemia often have low blood counts and require red blood cell and/or platelet transfusions, with associated risks including transfusion reactions and infections (such as HIV and hepatitis). Prior treatment may have weakened your immune system. It is possible that LMB-2 may also weaken your immune system. Infections that develop in

individuals with cancer can be very serious. You should seek immediate medical attention for fever greater than or equal to 100 degrees or any signs of infection.

Toxicity of filgrastim (G-CSF)
Filgrastim can cause skin rash, muscle and/or bone pain, an increase in any inflammatory conditions which you had before, like arthritis, enlarged spleen possibly causing a lowering of the platelet count, and abnormal blood chemistry levels. Loss of hair can occur with prolonged use.

Summary of severe toxicity of FC chemotherapy and antibody therapy:
- Likely (50-75% of patients): Low neutrophils
- Less Likely (25-50% of patients): Low platelets
- Unlikely (10-25% of patients): Infections including pneumonia

Risks Associated with Routine Procedures:
Blood Drawing: To monitor the effects of therapy frequent blood tests will be necessary. Up to a one unit (about 50 teaspoons) of blood, may be drawn every 4 weeks for research purposes while you are participating in the study. Every effort will be made to keep blood tests to a minimum. You will be monitored for anemia and given blood transfusions if needed. Side effects of blood draws include pain and bruising in the area where the needle was placed, lightheadedness, and rarely, fainting.

Bone Marrow Tests: If a bone marrow aspiration is done, your hipbone will be numbed with anesthesia, a small needle will be inserted into the hipbone, and about two tablespoons of bone marrow will be removed through the needle. This procedure usually causes only brief discomfort. Very rarely, infection or bleeding may occur at the needle site.

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

Ranitidine (Zantac): possible side effects include tiredness, dizziness, headache, and diarrhea.

Hydroxyzine (Atarax): Possible side effects include sleepiness, dizziness, restlessness, and irritability.
Patients infected with HIV will be excluded from this trial because the effect of LMB-2 on HIV replication and/or the immune system is unknown and potentially harmful. Patients with hepatitis C surface antigen positivity are excluded from this trial because the effect of LMB-2 on hepatitis C and/or the immune system is unknown and potentially harmful.

We will carefully monitor you to detect any of these side effects; in addition, you will be taught about side effects, which you may experience and must report immediately. Although side effects of this treatment usually last for a short period of time and completely resolve, you may experience side effects that are permanent. Although not expected, death could occur from this experimental treatment. It is very important that you notify us as soon as possible if you experience any type of side effect so that you can be carefully examined. All precautions will be taken to prevent these side effects and you will be treated promptly (if treatment is required and possible) if they occur. Treatment on this study will require a significant amount of your time and may be stressful. Participating in this study may prevent you from being in other research studies in the future.

Potential Benefits of Participation

While we hope that LMB-2 treatment will be beneficial to you, you may not benefit from this treatment. LMB-2 treatment may cause improvement in your leukemia such as reduction in cancer-related symptoms. Your participation in this study may help us advance the understanding of the use of biologic agents in the treatment of leukemia and lymphoma.

Research Subject's Rights

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.

- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Cancer Institute Institutional Review Board

A description of this clinical trial will be available on http://www.Clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest
- if your disease comes back during treatment
- if you have side effects from the treatment that your doctor thinks are too severe
- if new information shows that another treatment would be better for you

In this case, you will be informed of the reason therapy is being stopped.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to the Sponsor or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases can**not** be recalled and destroyed.

What Happens After This Treatment is Completed?

This depends on how you have responded to the experimental therapy. If you do not have evidence that the disease is worsening, we will schedule periodic visits to the Clinical Center for follow-up examination and tests. If the disease worsens then you may need other therapy. At that time you will be given the opportunity of participating in additional research protocols that may be appropriate for you. If no such protocols are available, you will be returned to the care of your local physician. It is important to stress that participation in this protocol does not constitute a promise of long-term medical care here at the Clinical Center. If there is no research study that is suitable for you and your stage of disease, you will be returned to the care of your private doctor or to a clinic in your local community. It is conceivable that participation in this study may make you ineligible to participate in certain other research protocols because the requirements for entry onto these protocols may disallow patients who have already been treated with certain drugs or who have had certain side effects from previous treatment. You may decide now not to receive treatment on this protocol, or you may choose at any point in time to stop the treatment and withdraw from the protocol; in either case you will be returned to the care of your referring physician.

Optional Studies (not required)

We would like to keep some of the specimens and data that are collected for future research. These specimens and data will not be identified by name when sent outside the NIH or stored, only by number. The use of your specimens and data will be for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimen and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you decide now that your specimens and data can be kept for research, you can change your mind at any time. Just contact us and let us know that you do not want us to use your specimens and/or data. Then any specimens that remain will be destroyed and your data will not be used for future research.

Please read each sentence below and think about your choice. After reading each sentence, circle and initial the answer that is right for you. No matter what you decide to do, it will not affect your care.

1. My specimens and data may be kept for use in research to learn about, prevent, or treat cancer or other health problems.

Yes No Initials_____

2. Someone may contact me in the future to ask permission to use my specimens and/or data in new research not included in this consent.

Yes No Initials_____

Optional Studies (not required)

- Soluble CD22, CD25, CD30, 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 may also be measured in your tumors if they need to be removed as part of your medical care, or if tumors can be easily and safely removed like with a skin biopsy.
- 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. HLA is the human leukocyte antigens, a complex of proteins on your white blood cells which allow your body to determine whether the cell is yours or not. 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. PAX-gene tubes contain a special liquid that keeps RNA in the blood stable, and it mixes with your blood only after it is drawn. Requires about 1/2 teaspoon.
- DNA samples to look for abnormalities which might make a patient more susceptible to toxicity. The genes to look at would include those that trigger cells to die, and those that help make hormones which cause inflammation. Requires about 1/2 teaspoon.
- Assays to identify genes in your DNA which would have significant risk to you, including how likely you or your children might be to get cancer, will not be done.
- 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. In flow cytometry, your blood after being drawn goes into a tiny tube where lasers determine whether the tumor markers are present if so how much. 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.

Disclosure of potential conflict of interest:

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The National Institutes of Health and the research team for this study have developed a drug, being used in this study. This means it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development of LMB-2.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Robert Kreitman, M.D., Building 37 Room 5124B, Telephone: 301-496-6947. Other researchers you may call are: Wyndham Wilson, M.D., Ph.D., Building 10; Telephone: 301-435-2415. You can contact either one through the hospital page operator 301-496-1211. You may also call the Clinical Center Patient Representative at 301-496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/ Legal Representative Date

Print Name

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian Date

Print Name

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian Date Print Name

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM DECEMBER 15, 2014 THROUGH JUNE 14, 2015.

Signature of Investigator Date

Print Name

Signature of Witness Date

Print Name