PROTOCOL SUBMISSION FORM **Amendment Form**	PROTOCOL NO. 04C0142-J	PRINCIPAL INVESTIGATOR (NIH Employee Name, Inst/Br, Telephone and e-mail): Robert Kreitman NCI LMB 301.496.6947 kreitmar@mail.nih.go

PROTOCOL TITLE: A Phase II Clinical Trial of Anti-Tac(Fv)-PE38 (LMB-2) Immunotoxin for Treatment of CD25 Positive Cutaneous T-Cell Lymphomas

ABBREVIATED TITLE: Treatment of CTCL w/LMB-2 Reference Number: 315879

Amendment Version Date: 06/14/2011 Amendment Letter: J

The Following revisions were incorporated into this protocol and approved by:

☒ Expedited Review (risk/ benefit ratio not changed)
☐ Full Board Review (meeting date)

Amendment includes changes required by:

☐ N/A
☒ NCI IRB (CCR)
☐ CTEP
☐ Other
☐ Other Sponsor
☐ FDA
☐ Special Studies IRB (DCEG)

If Other, list:

Amendment required Scientific Review?

☐ Yes ☒ No

ADMINISTRATIVE CHANGES

☐ Protocol Title/ Abbreviated Title
☐ New Principal Investigator
☒ NIH Personnel Change
☐ Non-NIH Personnel Change
☐ Converting to multi-institutional trial

DEC clearance required? ☒ Yes ☐ No

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PROTOCOL CONTENT CHANGES

☐ Precis
☐ Study Objectives
☐ Background and Rationale
☐ Eligibility Assessment and Enrollment
☐ Implementation of Study Design
☒ Supportive Care
☐ Accrual Ceiling Changed to: N/A
☐ Data Collection/Evaluation
☐ Human Subject Protections
☒ Data Reporting
☐ Ionizing Radiation Use
☐ Pharmaceutical Information
☐ Appendices

Does the amendment impact the risk/benefit assessment?

☐ Yes ☒ No

INFORMED CONSENT DOCUMENTATION

☐ Text Revisions to Consent(s)
☐ Investigator Contact Information on Consent(s)
☒ No Changes to Consent Form

SIGNATURE

Robert Kreitman - applied signature on 07/28/2011 7:00 PM EDT
New and Old Principal Investigators - electronic signature and date

APPROVALS

Ira Pastan - applied signature on 07/28/2011 8:18 PM EDT
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Not Applicable
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Giuseppe Giaccone - applied signature on 08/02/2011 1:35 PM EDT
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CTEP Protocol Number: 5943
Title of study: **A Phase II Clinical Trial of Anti-Tac(Fv)-PE38 (LMB-2) Immunotoxin for Treatment of CD25 Positive Cutaneous T-Cell Lymphomas**
Abbreviated Title: LMB-2 for CTCL

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PRECIS

Background:

It is estimated that 40-50% of patients with CTCL have tumors that express CD25 (Tac or IL2Ra). Normal resting T-cells do not express CD25. LMB-2 is an anti-CD25 recombinant immunotoxin containing variable domains of MAb anti-Tac and truncated Pseudomonas exotoxin. A phase I trial at NCI found that the MTD of LMB-2 was 40 µg/Kg IV given every other day for 3 doses (QOD x3) with prophylactic IV fluid. The most common adverse events were transient fever, hypoalbuminemia and transaminase elevations. In that trial, two of two patients with cutaneous T-cell lymphoma had clinical benefit (1 PR, 1 SD). In 1999 another recombinant fusion protein, denileukin diftitox, was approved by the FDA for treatment of patients with advanced or recurrent CTCL expressing the high affinity IL-2 receptor. This receptor is composed of three subunits: CD25, CD122 and CD132. Because LMB-2 is cytotoxic to cells expressing CD25 without the other IL-2 receptor subunits needed to form the high affinity receptor, CD25+ CTCL patients are good candidates for further testing with LMB-2.

Objectives:

The purpose of this study is to determine the activity of anti-Tac(Fv)-PE38 (LMB-2) in patients with Tac-expressing Cutaneous T-cell Lymphoma (CTCL). The primary endpoints of this trial are the response rate and response duration. We will also evaluate LMB-2 immunogenicity, pharmacokinetics, and toxicity, and monitor soluble Tac levels in the serum. These will be evaluated using routine hematologic and clinical evaluation, and when appropriate, by monitoring the phenotype of circulating T-cells or of biopsied tissues using antibodies to CD25.

Eligibility:

CD25+ CTCL based on immunohistochemistry or flow cytometry of blood. Patients must have measurable stage 1b-IV disease which progressed after $\geq$ 2 prior systemic or topical therapies. Labs required: ALT and AST $\leq$ 2.5-time upper limit, albumin $\geq$ 3, bilirubin $\leq$ 2.2, creatinine $\leq$ 2.0 (unless creatinine clearance $\geq$ 50 ml/min), ANC $\geq$ 1000/ul, and platelets $\geq$ 50,000/ul (ANC and platelets $\geq$ 500 and 10,000 if blood/marrow involvement).

Design:

Patients receive LMB-2 30 ug/Kg QOD x3 every 4 weeks in absence of neutralizing antibodies or progressive disease. Dose escalation to 40 ug/Kg QOD x3 if < 2/6 DLT at 30 ug/Kg x3. 1st stage is 16 patients, to expand to 25 if > 1 of 16 patients respond.

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1 INTRODUCTION

1.1 OBJECTIVES OF THE TRIAL:

1.1.1 Primary:

- To study the response rate and response duration in patients with CD25-positive cutaneous T-cell lymphoma treated with LMB-2.

1.1.2 Secondary:

- To describe the relationship between immunogenicity, toxicity, and serum concentrations of LMB-2.
- To determine if soluble Tac-peptide (sIL2Rα) levels correlate with response to treatment with LMB-2.

1.2 BACKGROUND AND RATIONALE:

1.2.1 Cutaneous T-Cell Lymphoma

Cutaneous T-cell lymphomas (CTCL) are a group of lymphoproliferative disorders characterized by malignant CD4+(usually) T-lymphocytes which localize to the skin on initial presentation. Mycosis Fungoides and Sezary syndrome (MF/SS) make up the great majority of these diseases. For review, see references 1-3. MF/SS accounts for 2% of new cases of NHL. The incidence of MF/SS in the general population is 3 to 4 per million with 1000-1500 new cases and around 500 deaths per year. There is a 2:1 male predominance. It commonly affects older adults (median age 55) and it may be more common in African-Americans (4).

Clinical presentation, histology, immunophenotyping and gene rearrangement studies are important in the initial evaluation of MF/SS. The most common immunophenotype is CD3+, CD4+, CD45RO+, CD7-. CD25 expression is present in 40-50% of CTCL patients (5-7). Patients with MF typically present with pruritic, mildly erythematous, scaly patches or plaques in non-sun-exposed areas. Plaques may eventually develop into ulcerating or fungating tumors which often become infected. Over time, MF spreads to the regional lymphatics and viscera. The organs most commonly involved are the spleen, liver, and lungs.

SS, which accounts for 5% of new presentations of MF/SS, presents with generalized erythroderma, pruritis, and circulating abnormal cerebriform lymphocytes called Sezary cells (8). Although the presence of Sezary cells in the blood is not included in the clinical staging, it usually correlates with more advanced T-stage (usually T4) and the presence of extracutaneous disease. Bone marrow involvement is rare in early stages but is usually present in SS. Staging of MF is noted below (1, 9).

TNMB Classification for MF/SS

T1 Limited patch/plaque (<10% of total skin surface)
T2 Generalized patch/plaque (>10% of total skin surface)
T3 Tumors (tumor mass raised ≥5 mm above normal skin)
T4 Generalized erythroderma

N0 No Clinically abnormal peripheral lymph nodes
N1 Clinically abnormal peripheral lymph nodes
NP0 Biopsy performed, not CTCL
NP1 Biopsy performed, CTCL

M0 No visceral metastases
M1 Visceral metastases

B0 Blood has no Sezary cells (<5% of lymphocytes)
B1 Blood has Sezary cells (>5% of lymphocytes)

Stage Classification for MF/SS

Stage	TNM Classification	
IA	T1	
IB	T2	
IIA	T1-2	N1NP0
IIB	T3	N0NP0
IIIA	T4	N0
IIIB	T4	N1NP0
IVA	T1-4	NP1 (N0 or N1)
IVB	T1-4	N0-3 M1

* The Blood Classification does not alter clinical stage

On initial presentation 36% of CTCL patients have T1 disease, 40% have T2, 10% have T3 and 14% are T4. Patients with stage IA disease rarely progress (only 9%) and have normal life expectancies. Twenty-four percent of patients with T2 (stage IB/IIA) have progressive disease and 20% die of causes related to MF. Median survival is greater than 11 years. The majority of patients with stage III and IV disease die of MF with median survivals of 3.2 and 4.6 years, respectively (2).

Treatment of this chronic incurable lymphoma is oriented towards symptom relief, cosmetic improvement, and prevention of disease progression and secondary complications, which in turn have an impact on survival (1, 10). Skin-directed treatment is given to all patients with MF; but as the disease progresses and becomes refractory to those therapies, systemic treatment is employed. Skin-directed therapy includes topical steroids, psoralens plus ultraviolet light (PUVA), topical chemotherapy using nitrogen mustard or BCNU, and radiation therapy.

Systemic therapy includes chemotherapy, photopheresis, α-interferon (IFN-α), retinoids, and denileukin diftitox, and combinations of these therapies with each other or with topical therapies. The most effective combination chemotherapy regimens are CHOP

and EPOCH which have up to 80% response rates (25-30% CR rates); however, the median response duration is less than 1 year (11). In addition, these patients trade significant toxicities for little to no improvement in overall survival. Single agent chemotherapy regimens using fludarabine, 2'-deoxycoformycin, etoposide, methotrexate, bleomycin, or vinblastine have less toxicity but have 40-50% response rates (12, 13). Extracorporeal photopheresis (ECP), bexarotene (Targretin™, oral and topical), and denileukin diftitox (Ontak™) are the only three FDA-approved therapies for CTCL.

ECP induces response rates of 50-75% with some evidence that it may prolong survival in a select group of SS patients with low tumor burden (14). The FDA approved Bexarotene, a retinoid that selectively activates retinoids X receptors, in December 1999 after showing a 27% response rate based on the Composite Assessment of Index Lesion Disease Severity of 56 patients (15). The median time to response was 180 days and the projected median time to relapse was 299 days for the group treated at 300 mg/m2/d. The toxicities, including hypertriglyceridemia, thyroid abnormalities, and leukopenia, were reversible with discontinuation of the drug (15).

Denileukin diftitox, a recombinant fusion protein of diphtheria toxin fragments A and B linked to interleukin-2 (IL-2), targets the high affinity IL-2 receptor which is composed of three subunits: CD25, CD122 and CD132. It was approved for advanced or recurrent CTCL by the FDA in February 1999 based on a 30% response rate (10% CR, 20% PR) in 71 patients lasting an average of 4 months (7). Eligibility required ≥20% expression of CD25 on the lymphocytes in the skin. One hundred twenty-five (45%) of the 276 CTCL patients screened on this trial met this requirement. The drug was given as a 15-60 minute bolus infusion daily for 5 days about every 21 days up to 8 cycles or 6 months regardless of antibody production which may have limited response. Up to 3 additional cycles could be given to patients who had an ongoing response. The median time to response was 6 weeks and the median duration of response was 6.9 months. Prior to therapy, 32% of patients had anti-diphtheria toxin antibodies which cross-reacted with denileukin diftitox in an ELISA assay. After 1 cycle, 63/71 patients developed antibodies; after 2 cycles, 70/71 patients developed antibodies. An in vitro cell-based assay demonstrated the neutralizing activity of these antibodies. The clearance of the drug increased significantly with rising antibody levels; however, "some patients experienced significant antitumor effects that evolved over time during the treatment period" (7). In addition, higher neutralizing antibody levels were associated with a lower incidence of transaminase elevation, hypoalbuminemia, and rash. Elevated antibody levels did not correlate with acute infusion-related events or vascular leak syndrome. Toxicities included acute hypersensitivity-type reactions, constitutional symptoms, infections, and vascular leak syndrome (7).

1.2.2 Targeted Toxins and Antibody-based Therapies

Antibody-based therapies have become widely accepted since the FDA approved Rituximab in 1997 after showing significant clinical activity in patients with low-grade lymphoma. Other unconjugated monoclonal antibodies, trastuzumab and CAMPATH-1, are now FDA approved therapies for advanced breast cancer and CLL. In 1997 the FDA approved daclizumab (Zenapax™), an anti-CD25 antibody, for the prophylaxis of

acute organ rejection in patients receiving renal transplants. (Chronic T-cell activation from a transplanted organ up-regulates CD25). The radioimmunoconjugates (RICs) ^{131}I tositumomab and ^{90}Y Ibritumomab have recently completed trials showing efficacy in CD20+ lymphomas and have been approved by the FDA (16, 17). Denileukin diftitox, the only FDA-approved tumor-targeted toxin, has demonstrated efficacy in CTCL and is currently being tested in indolent lymphomas in three phase II multi-institutional trials.

Immunotoxins are immunoconjugates that couple the specificity of a monoclonal antibody (Mab) with a highly lethal cellular toxin. Toxins are proteins produced by plants or bacteria which internalize into animal cells and kill them by inhibition of protein synthesis. Plant toxins inhibit protein synthesis by inactivating ribosomes (18), while bacterial toxins including *Pseudomonas* exotoxin (PE) and diphtheria toxin, inactivate elongation factor 2 (EF2) (19, 20). Toxins are active in minute quantities because they function enzymatically and in fact only one molecule in the cytoplasm is sufficient to kill cells (18, 21). Plant toxins include ricin A chain (RTA), a form of RTA which is deglycosylated to prevent binding to liver (dgA), full-length but mutated "blocked" ricin, pokeweed antiviral protein (PAP), and Saporin. Bacterial toxins administered clinically included Pseudomonas exotoxin (PE) and diphtheria toxin (DT). These toxins usually contain two or three chains (domains). One of these chains facilitates cell binding. An immunotoxin is created when an antibody-binding domain is substituted for the cell binding chain of the toxin.

BL22, an anti-CD22 (dsFv)-PE38 immunotoxin, has been tested in chemotherapy-refractory hairy cell leukemia patients over the last 2-½ years at the National Cancer Institute (22). Twenty-five HCL patients completed 1-17 cycles of BL22 at dose levels of 10-50 µg/Kg every other day for 3 doses (QOD x3). Twenty-one of the 25 patients responded (18 CR, 3 PR). The 4 without major responses had low doses, neutralizing antibodies, or massive lymph nodes. CRs were induced after 1 cycle in 11 patients and after 2-13 cycles in 7 patients. With a median follow-up of 12 months (range 2-24 months), 4 patients relapsed, of which 3 achieved a second CR with additional BL22 treatment, and 1 with an asymptomatic marrow relapse was not retreated. To prevent relapse, patients received up to 4 repeat cycles of BL22 at 3-5 week intervals after achieving CR. Of the 40 patients treated on that trial, 5 developed HUS. The first patient (received 1 cycle) had an aggressive B-cell lymphoma and eventually died with progressive disease without resolving her kidney dysfunction. The other 4 patients (2 with 2 cycles and 2 with 3 cycles, all 3 weeks apart) had HCL and were treated with plasmapheresis with complete resolution of symptoms and lab abnormalities (in 15, 21, 41, and 73 days). Three of those patients had complete remissions and the 4th had complete resolution of her cytopenias and clearing of her blood and bone marrow (a retroperitoneal LN remained).

1.2.3 LMB-2 development

1.2.3.1 Anti-Tac

One strategy to enable a monoclonal antibody to kill cells is to use the antibody to block a growth factor receptor. IL-2 is a growth factor for antigen-stimulated T cells and is responsible for T cell clonal expansion after antigen recognition. It is primarily produced by CD4+ T cells. The high-affinity IL-2 receptor (IL-2R) consists of 3 noncovalently associated proteins called α (CD25), β (CD122), and γ (CD132), the latter two being members of the type I cytokine receptor family. The α and β chains are involved in cytokine binding, and the β and γ chains are involved in signal transduction. The IL-2Rα, originally called the Tac antigen (for T cell activation), is a 55-kD polypeptide that normally appears when T cells are activated. It is usually not expressed on resting T-cells, B-cells or monocytes (23). However, IL-2R$\beta\gamma$ (low-affinity IL-2R) is expressed on resting (naïve) T cells and binds IL-2 at 1 x 10 (-9) M concentration. When T cells are stimulated and produce α chains the affinity of IL-2 increases to 1 x 10 (-11) M. Chronic T cell stimulation can lead to the shedding of a 45-kD piece of the IL-2Rα into the blood. The serum soluble Tac (sIL-2Rα) level can then be followed as a marker of strong antigenic stimulation such as in rejection of a transplanted organ (24).

Anti-Tac is a monoclonal antibody which binds to IL2Rα (CD25) with high affinity blocking the interaction of IL2 with the IL2R (25-29). Certain malignant cells constitutively express large numbers of the CD25 receptors. As noted above, 40-50% of CTCL patients express CD25. Other tumors that can express CD25 include Adult T cell leukemia/lymphoma (25, 30-32), peripheral T-cell leukemia/lymphomas (33, 34), hairy cell leukemia (35, 36), chronic lymphocytic leukemia (37-39), and lymphomas (6, 40-42). In these malignancies, soluble Tac is often present and may serve as a good surrogate for tumor response and progression (43-47).

Unmodified humanized anti-Tac monoclonal antibody (daclizumab, NSC-277524, IND #2091) was tested in 19 ATL patients (NIH 83-C-0023) to determine if it would block IL2 from stimulating the malignant cells (48, 49). There was no significant toxicity. Six patients had remissions including 4 partial and 2 complete remissions lasting from one to more than 43 months after onset of therapy. Although this approach to the treatment of ATL was encouraging, 13 patients did not respond. Furthermore, 5 of the responding patients relapsed following therapy. Relapses were not due to loss of IL2Rα expression by the ATL cells, but rather to loss of dependence of the ATL cells on IL2 for their proliferation. Thus, since IL2Rα is still expressed on the malignant cells, it remains a target for immunotherapy.

1.2.3.2 PE38

The full-length 613 amino acid PE protein contains three functional domains which are necessary for cellular intoxication (50, 51). 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 contains the following steps: 1) The C-terminal residue (lysine-613) is

removed by a carboxypeptidase in the plasma or culture medium (52). 2) Domain Ia binds to the α2 macroglobulin receptor, present on animal cells (53). 3) After internalization at low pH, domain II is proteolytically cleaved between amino acids 279 and 280 by furin (54-56). 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 (57, 58). 6) Amino acids 280-313 mediate translocation of the toxin to the cytosol (59, 60). 7) The ADP ribosylating enzyme composed of amino acids 400-602 inactivates EF-2 (19). 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 (50, 61). PE38 is a truncated version of PE40 which is missing amino acids 365-380. While PE40 and PE38 have similar activities (62, 63), we have preferred to use PE38 because it is slightly smaller and is missing a disulfide bond that impairs refolding the protein.

1.2.3.3 The Recombinant Immunotoxin

The initial approach was to produce a chemically conjugated immunotoxin comprised of murine anti-Tac and the entire *Pseudomonas* exotoxin molecule that was treated with iminothiolane to decrease its native cellular toxicity (64) and facilitate conjugation to anti-Tac. *In vitro* studies of inhibition of protein synthesis in an HTLV-I positive T-cell line HUT-102 showed that protein synthesis was inhibited by 50% (IC_{50}) with 1.2 ng/ml (5 pM), compared to 90-880 ng/ml for IL2Rα-negative cells (61). From June 1985 through November 1987, 5 patients with T-cell leukemia were treated with anti-Tac-PE. The patients were treated intravenously with doses of 0.2 to 2.0 mg for a total dose of up to 4.4 mg over 1 week. Two of the five patients developed Grade III or IV hepatotoxicity with one manifesting a transient elevation of the hepatic transaminases (SGOT & SGPT) to levels of 1000-1200 units/l and the other patient having a rise in the bilirubin from normal to 4.1 mg/dl. Neither of these reactions was associated with clinical symptoms and in both patients the abnormalities remitted within two weeks of stopping treatment. None of the five patients manifested a clinically apparent tumor response.

In an attempt to improve the therapeutic index and pharmacokinetics of the immunotoxin, alterations have been made to both the antibody binding portion and the toxin molecule. The non-specific toxicity of the toxin was dramatically reduced by removing domain Ia of the binding domain (61). The chemical conjugate (anti-Tac-Lys-PE40) was less cytotoxic than anti-Tac-PE toward HUT-102 cells, with an IC_{50} of 13 pM compared to 5 pM, but the non-specific cytotoxicity toward non-IL2Ra-bearing cells improved 6 to 100-fold (61). To make an anti-IL2Ra toxin in single-chain form which would not require chemical treatment of the toxin, human IL2 was fused to PE40 and the resulting IL2-PE40 purified from *E. coli* was cytotoxic to cells with an IC_{50} of 5 pM (65). However, IL2-PE40 and the more active derivative IL2-$PE66^{4Glu}$ showed insufficient cytotoxicity toward activated human T-lymphocytes (66).

To target cells with a single-chain protein containing the antigen binding domains of anti-Tac, the variable domains of the antibody (V_H and V_L) were fused together with the peptide linker $(G_4S)_3$ and the resulting Fv fragment of anti-Tac was fused to PE40 (67).

Anti-Tac(Fv)-PE40 was extremely cytotoxic with an IC_{50} of 0.15 ng/ml toward HUT-102 cells (67).and 0.05-0.1 ng/ml toward activated human T-cells (63, 68). To determine if malignant cells in patients have enough receptors and metabolize the toxin effectively enough to be sensitive to anti-Tac(Fv)-PE40, we tested ATL cells from the blood of 38 patients and from the lymph nodes of 5 patients. All samples were sensitive to anti-Tac(Fv)-PE40, with IC_{50}'s of 0.03-16 ng/ml (63, 69-71). Anti-Tac(Fv)-PE40 was shortened slightly by removing amino acids 365-380, resulting in anti-Tac(Fv)-PE38 (LMB-2). The cytotoxic activity of anti-Tac(Fv)-PE38 (LMB-2) appears identical to that of anti-Tac(Fv)-PE40 toward cell lines and fresh ATL samples (63). Importantly, it was shown that these recombinant anti-IL2Rα–immunotoxins are still cytotoxic in the presence of soluble IL2Rα concentrations as high as those expected in ATL patients (71). This suggested that even in ATL patients, whose sera have the highest levels of soluble IL2Rα, anti-Tac(Fv)-PE38 (LMB-2) should not be prevented by soluble IL2Ra from reaching the malignant cells.

1.2.3.4 Preclinical studies of LMB-2

A mouse model of a human IL2Rα positive malignancy was produced by the subcutaneous injection in nude mice of ATAC-4 cells (72). These cells are A431 epidermoid carcinoma cells that have been transfected with the gene encoding IL2Rα, and contain 2 x 10^5 IL2Rα sites/cell (63). Mice began treatment with LMB-2 four days after ATAC-4 cell injection, when subcutaneous tumors became established (32-86 mm^3). Ninety-100% tumor regressions were observed in 2 of 5 mice receiving 30 µg/Kg i.v. QD X 3, and in 5 of 5 mice receiving 60 µg/Kg i.v. QD X 3. These doses were respectively 5 and 10% of the mouse LD_{50} (72). When administered to mice every other day, complete tumor regressions could be obtained in 5 of 5 mice receiving 100 µg/Kg i.v. days 4, 6 and 8. The LD_{10} and LD_{50} are 200 and 257 µg/Kg every other day for 3 doses. The cause of death on necropsy of the mice was liver damage.

Cynomolgus monkeys were used to determine the safety and pharmacokinetics of LMB-2, since anti-Tac reacts with primate but not murine IL2Rα. In a GLP pharmacokinetic study, the elimination of LMB-2 from the serum followed biphasic kinetics, with a $T_{1/2}\alpha$ of 45 minutes and a $T_{1/2}\beta$ of 127 minutes. In a GLP toxicology study, 4 Cynomolgus monkeys received 20 µg/Kg days 1, 3 and 5 with no significant toxicity. Four monkeys were then given 300 µg/Kg days 1, 3 and 5 and experienced dose-limiting toxicity with anorexia and 2 to 4-fold transaminase elevations.

One of two monkeys (male) autopsied on day 7 in this high-dose group had hepatomegaly, mild diffuse hepatocyte vacuolation, testicular and epididymal degeneration, and leukocytosis and vascular inflammation of the skin. The other monkey (female) had a ~0.5 X 0.8 mm area of myocardial degeneration. Of the four high-dose monkeys, the one with hepatocyte vacuolation had the mildest (up to 2-fold) transaminase elevations. The two remaining high-dose monkeys were sacrificed on day 51. One of these (male) was found to have a ~0.4 X 0.4 mm area of myocardial degeneration and testicular and epididymal degeneration. The other (female) was found to have a myocardial parasite. No monkeys had detectable CPK-MB positivity or LDH I/II isoenzyme ratio greater than one at any time point. After the toxicology study was completed, it was reported by a group at Parke-Davis Research Institute that

untreated wild-caught Cynomolgus monkeys have high rates of various pathologic lesions. In particular, of 62 male and 62 female animals, 45.2% of males and 48.4% of females had cardiac lesions, 58.1% of males and 54.8% of females had hepatic lesions, 31.4% had skin lesions and 13% of males had immature testicular and accessory sex organ histology (79). Unpublished data from these investigators indicated that 6.4% of the males and 14.5% of the females had myocardial degeneration and/or necrosis, 8.1% of the males and 3.2% of the females had hepatic necrosis, 4.8% of males and 1.6% of females had hepatic vacuolation, 6.4% of males and females had mixed cell infiltrates in skin, 1.6% of males and 3.2% of females had inflammation in the skin and 12.9% of males had immature testes.

In summary, the GLP monkey toxicology study of anti-Tac(Fv)-PE38 (LMB-2) showed that the dose limiting toxicity was due to hepatic toxicity, manifested by transient transaminase elevations and loss of appetite in monkeys receiving the 300 µg/Kg X 3 dose. Based on the inconsistent appearance of pathologic lesions, their lack of correlation to laboratory abnormalities, and the reported high prevalence of similar lesions in untreated wild-caught Cynomolgus monkeys, the hepatic vacuolation and the testicular, epididymal and myocardial degeneration may have been unrelated to the anti-Tac(Fv)-PE38 (LMB-2) administered. Most importantly, anti-Tac(Fv)-PE38 (LMB-2) at a dose of 20 µg/Kg X 3 was shown to have no significant toxic effects in all 4 monkeys tested.

1.2.3.5 Phase I trial of LMB-2 (NIH #96-C-0064)

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 (73). 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. Twenty patients developed anti-PE38 neutralizing antibodies and 9 of those patients also developed HAMA antibodies. Twenty-nine of the 35 patients received a starting dose of ≥ 10µg/Kg QOD x 3.

LMB-2 related toxicity with at least one cycle of LMB-2 was seen at ≥ 10µg/Kg QOD x 3 and was reversible. Please see table 1.2.3.5.1 below for a list of toxicities for the first 35 patients. The common toxicity criteria (CTC) of the NCI were used to grade toxicity. Dose Limiting Toxicity (DLT) was defined as at least grade 3 toxicity (attribute possible, probable, or definite), but the following exceptions were not considered to be dose-limiting: (1) transaminases elevations of 5-20 times normal, (2) bilirubin 1.5-2.2 times normal, (3) fever that was well tolerated and did not result in a break in therapy, (4) hematologic toxicity in leukemic patients, (5) grade 3 hematologic toxicity in nonleukemic patients, and (6) abnormal coagulation profiles in patients who were receiving anticoagulant therapy or who had preexisting coagulation abnormalities.

DLT was observed in two of three patients at the 63 µg/Kg QOD x 3 dose level. The first patient had asymptomatic grade 4 AST and grade 3 ALT elevations. The second patient (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 either cytokine mediated or due to direct LMB-2 toxicity on the heart. The 50 µg/Kg dose level was dose limiting in 1 of 6 patients due to an allergic reaction.

Four of the five patients who had incomplete cycles had LMB-2-related toxicities (2 allergic reactions and 2 DLT at the maximum dose; the fifth patient was stopped after culture-positive influenza B pneumonia after the first dose of cycle 1). The most common toxicities were transient fever and transaminase (ALT, AST) elevations, usually grade 1 or 2. Twenty-six of 29 patients (90%) had transaminase elevations. Grade 3 elevations were observed in 1, 3, and 2 patients at dose levels of 30, 40, 50 µg/Kg QOD x 3, respectively, but were not associated with impaired liver function as assessed by PT, bilirubin, and fibrinogen levels. Several patients receiving 40-63 µg/Kg QOD x 3 experienced transient grade 1 nausea and vomiting, but this could not be correlated with the degree of transaminase elevations. Patients did not have evidence of cumulative hepatic toxicity with retreatment; and in all cases transaminase elevations resolved to pretreatment levels before patients began a subsequent cycle. The transaminase elevations were never observed to increase after day 8 of each cycle in any patient. In all patients with grade 3-4 elevations, the AST and ALT levels resolved to ≤40 U/L. Therefore, we decided that grade 4, not grade 3, transaminase elevations would be dose limiting.

Fever (22/29 patients) typically occurred within hours of the first dose and did not recur after the second and third doses. Often it recurred on subsequent cycles after the first dose. Eighteen of 22 patients with normal pretreatment albumin levels (≥3.7g/dL) experienced hypoalbuminemia, 11 patients with grade 1 and 7 with grade 2. Most of these patients did not develop significant weight gain and none had symptomatic pulmonary edema. Reversible grade 1 drug-related renal toxicity was observed in 3 patients. In patients treated at the MTD (40 µg/kg), the mean Cmax values were 360 ng/ml with mean half-lives of 280 minutes.

The MTD (40 µg/Kg QOD x3) was well tolerated by all 9 patients treated with only transient toxicity (see table 2 in section 1.2.3.5.2 below). All toxicities were mild (grades 1-2) with the exception of grade 3 transaminase elevation (3) and thrombocytopenia (1).

Four additional patients have been treated with LMB-2 using prophylactic i.v. fluid, i.e. 2 ml/Kg/hr from 4 hours before to 18 hours after each dose, to determine if improved hydration could decrease the cytokine release syndrome. These patients were all graded by CTC 2.0.

Patient #36 with ATL was treated at 50 µg/Kg QOD x3 and experienced a reversible but dose-limiting syndrome similar to patient #30, with 3rd spacing and muscle edema causing grade IV CK elevation and hypoventilation leading to supraventricular tachycardia and respiratory failure. Associated toxicity included grade III hypoalbuminemia, grade II ALT, grade III AST, grade II acidosis, grade I fever, grade I GGTP, grade II lipase, grade I thrombocytopenia grade III hypotension and grade II weight gain. 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. Due to this DLT event, the dose level was reduced to 40 µg/Kg QOD x3.

The 3 patients treated at 40 µg/Kg QOD x3 with prophylactic fluid had HD (#37), CTCL (#38) and NHL (#39), and did not have DLT. Patient #37 experienced grade II hypoalbuminemia, grade I ALT and AST, grade II fever, grade I nausea, grade I thrombocytopenia, and grade I weight gain. Patient #38 had grade II hypoalbuminemia, grade I ALT and AST, grade I fever, and grade I myalgia. Patient #39 had grade II hypoalbuminemia, grade I AST, grade I creatinine, grade II diarrhea, grade II edema, grade I fever, grade II fatigue, grade II nausea, grade II myalgia, grade I pleural effusion, grade I hematuria, and grade II weight gain.

One of the two patients with CTCL had a PR. She had Sezary syndrome (stage IVa) with erythroderma, pruritis, and a circulating Sezary count of 1000 cells/ul. She responded to the first cycle of LMB-2 with a 97% reduction of Sezary cells by day 3 and regression of symptomatic disease. The response was maintained > 6 months with 6 repeated cycles of LMB-2 (cycles 1-4 at 30µg/Kg , cycles 5-6 at 40µg/Kg). Ex vivo incubation of the patient's peripheral blood mononuclear cells (containing 50% Sezary cells) with LMB-2 resulted in a maximum protein synthesis inhibition of 33% with half-maximal inhibition at 0.2 +/- 0.09 ng/ml. This was comparable to the IC_{50}s of LMB-2 toward hairy cells, and ~0.1% of plasma levels of LMB-2 achieved in patients. Cytotoxicity was specific for CD25 and not produced by BL22, an anti-CD22 immunotoxin.

The second CTCL patient was recently treated at 40µg/Kg and has had stable disease for over three months. He had stage IIb disease prior to LMB-2 and 8 years of pruritis throughout previous therapies. By day 3 of cycle 1 he had resolution of his pruritis. By day 8, his only tumor flattened to a plaque (making him a stage Ib), all other lesions decreased in erythema, size and scaling, and he had a 50% clearing of malignant cells by skin biopsy. Unfortunately he developed neutralizing antibodies during cycle 2 and is therefore not eligible for further treatment (based on our current protocol). The Cmax values of LMB-2 were 300-450 ng/ml with half-lives of 206-228 minutes in the CTCL patients.

In summary, LMB-2 is well tolerated at the MTD. The most common toxicity, transaminase elevation, was reversible and never associated with any other evidence of hepatic dysfunction. All other toxicities at the MTD were also transient, resolving within a few days to weeks after treatment.

1.2.3.5.1 Table 1 (73)

LMB-2-related toxicities for patients treated at ≥10μg/Kg QOD x 3 (patients 7-35)

Toxicity	**Dose Level: Total patients (total Grade 3 or 4)**					
	10	**20**	**30**	**40**	**50**	**63**
Total treated	3	3	5	9	6	3
Transaminases	2	2	5(1)	8(3)	6(2)	3(2)
Fever	0	2	5	7	5(1)	3(1)
Alkaline phosphatase	0	0	0	3	0	3
Thrombocytopenia	0	1	0	2(1)	1	1
Hypoalbuminemia	1	1	4	6	3	2
Hypotension	0	0	0	3	3	2
Nausea/Vomiting	0	0	0	5	2	3
Diarrhea	0	0	0	0	0	1(1)
Pericardial effusion	0	1	1	2	0	2
Weight Gain	1	0	1	2	1	2
Allergy	0	0	0	1	2(1)	0
Increased Creatinine	0	0	0	2	1	0
Proteinuria	0	0	0	2	1	0
Cardiomyopathy	0	0	0	0	0	1(1)
Total Evaluated	3	3	5	9	6	3

1.2.3.5.2 Table 2 (73)

LMB-2-related toxicities for the first 9 patients treated at the MTD (40 μg/Kg)

Toxicity	**Grade**			**Total # of patients**
	1	**2**	**3**	
Transaminases	3	2	3	8
Fever	1	6		7
Alkaline phos	2	1		3
Thrombocytopenia	1		1	2
Hypoalbuminemia	2	4		6
Hypotension		3		3
Nausea	4	1		5
Vomiting	1	1		2
Creatinine/Proteinuria	2			2
Pericardial effusion	2			2
Weight Gain	2			2

1.2.3.6 LMB2 Test Dose

All patients on the Phase I trial received a 10 μg test dose before the first dose of LMB2 on day 1 of each cycle. The test dose was administered 30 minutes prior to the LMB2 treatment dose which was 30 minutes after pre-medication. There was no correlation between patient tolerance of test doses and LMB2 treatment. Notably, no patients were taken off study due to test dose reactions. Furthermore, none of the patients who had

allergic toxicity had sustained reactions to the test dose. Due to this lack of utility, test doses will not be administered on this trial.

1.2.4 Rationale of Study Design

Because most patients with CTCL become refractory to current treatment options, new therapies are needed. Based on the efficacy of the BL22 immunotoxin in HCL, the clinical benefit of LMB-2 in two of two patients with CTCL, and the success of denileukin diftitox in CTCL, it is reasonable to pursue a phase II trial testing LMB-2 at the established MTD in CTCL patients. In addition, patients who have failed denileukin diftitox may still respond to LMB-2 because it is cytotoxic to cells expressing CD25 without the other IL-2 receptor subunits needed to form the high affinity receptor required by the denileukin trial. Failure of response to denileukin due to neutralizing antibodies does not predict response to LMB-2 because it has different antigenic epitopes. Similarly to the denileukin trial, LMB-2 will be given every 4 weeks for up to 6 cycles in the absence of neutralizing antibody production; and up to 3 additional cycles can be given to patients who have a documented ongoing response between cycles 4 and 6 (7).

1.2.5 Dose Determination

The MTD established in the phase I trial of LMB-2 will be used to treat patients on this trial. This dose is 40 µg/kg IV given every other day for 3 doses combined with prophylactic IV fluid. Every other day dosing will be used so that toxicity can be observed prior to giving the second and third doses.

1.2.6 Prophylactic Dexamethasone

Several patients treated with LMB-2 have developed completely reversible allergic reactions other than rash, associated with immunogenicity to LMB-2. Immunogenicity to the toxin domains of similar immunotoxins BL22, HA22 and SS1P is not associated with allergic reactions other than rash, suggesting relationship to the targeting domain, anti-Tac(Fv). Grade 3-4 allergic reactions observed during phase I testing included grade 3 bronchospasm during the 1st dose of cycle 2 in a patient with HD, and grade 4 anaphylaxis during the 1st dose of cycle 1 in a patient with PTCL (73). The patient with anayphylaxis during cycle 1 of LMB-2 had prior humanized anti-Tac Mab (Zenapax, daclizumab) treatment and was in retrospect found to have human anti-human IgG antibody (HAHA), suggesting that the idiotype of LMB-2, which is also present in humanized anti-Tac Mab, plays a role in both immunogenicity and allergic reactions. Of 9 CTCL patients who have undergone phase 1 or 2 testing, one had an allergic reaction during cydle 2, associated with an unusual feeling in the throat. Of 11 HCL patients who have undergone phase 1-2 testing, 2 had syncopal episodes during retreatment, one of whom noted an identical episode with rituximab which was subsequently prevented with steroids. The level of neutralizing antibodies prior to the cycle causing allergic reactions was either low or undetectable and did not correlate with allergic reaction. To prevent these allergic reactions, CTCL patients will receive steroid prophylaxis with a single dose of dexamethasone 20 mg i.v. 2 hours prior to the first dose of each retreatment cycle of LMB-2.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA:

2.1.1 Inclusion Criteria

- Patients must have histopathological evidence of CD25+ CTCL confirmed by the NIH pathology department. One of the following must be present:
  - ≥20% expression of CD25 on the lymphocytes in the skin at a site of a patch, plaque, or tumor.
  - ≥20% of the peripheral blood Sezary cells must be CD25+.
- Measurable stage Ib-IV disease that has progressed after at least 2 prior systemic or topical therapies.
- Patients must have a ECOG performance status of 0 - 2 and be at least 18 years old.
- Patients must be able to understand and give informed consent.
- Patients must be 4weeks from any monoclonal antibodies.
- Patients must be ≥3 weeks from any CTCL-specific therapy and have evidence of progressive disease. Patients who are on chronic steroids must be on a stable dose of Prednisone ≤20 mg/day (or equivalent dose of another steroid) for at least 3 weeks and have evidence of progressive disease.
- Female patients of childbearing potential must have a negative pregnancy test and must use effective contraception (a barrier form of contraception).
- The transaminases ALT and AST must each be ≤2.5-times the upper limits of normal. Albumin must be ≥3.0 gm/dL. Total bilirubin must be ≤2.2 mg/dL.
- The creatinine must be ≤2.0 mg/dL or the creatinine clearance must be ≥50 ml/min.
- The ANC must be ≥ 1000/mm^3 and the unsupported platelet count must be ≥ 50,000/mm^3 in patients without blood or bone marrow involvement. If there is blood or bone marrow involvement, the ANC must be ≥500 mm^3 and the platelets must be ≥10,000/mm^3.
- The cardiac ejection fraction as assessed by echocardiogram or nuclear medicine study must not be less than the institutional limit of normal.
- Pulmonary function studies must demonstrate a DLCO ≥ 55% and a FEV1 ≥ 60% of normal for inclusion

2.1.2 Exclusion Criteria

- Patients whose serum neutralizes LMB-2 in tissue culture, due either to anti-toxin or anti-mouse-IgG antibodies. No patient whose serum neutralizes > 75% of the activity of 1 µg/mL of LMB-2 will be treated.
- Patients who are pregnant or breast-feeding.

- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Patients who are HIV positive, hepatitis B antigen positive, hepatitis C PCR positive, or who have other chronic liver disease (see section 6.1.3).
- Patients with symptomatic cardiac or pulmonary disease.
- Patients on warfarin therapy. Such patients may be eligible if they can be switched to heparin or low-molecular weight heparin therapy and are off warfarin at least 4 days prior to study enrollment.
- Active cancer requiring treatment.

2.2 RESEARCH ELIGIBILITY EVALUATION

- Evidence of CD25 expression must be obtained no earlier than a year prior to enrollment.
- A tumor sample will be submitted to the NIH pathology department for analysis of CD25 expression (an eligibility criterion). This analysis will be done in the Laboratory of Pathology, NIH (Anatomic Pathology Dept., CC), a College of American Pathologists (CAP) and CLIA-approved laboratory, by standardized diagnostic techniques, including immunohistochemistry and flow cytometry. Use freshly frozen tumor tissue (preferred) or paraffin-embedded tissue. Tumor blocks obtained at outside institutions should be shipped to:

  Linda Ellison R.N.
  NCI, Building 10, Room 12N214
  9000 Rockville Pike
  Bethesda, MD 20892
  Tel: 301-496-9458
- Complete history and physical examination with documentation of measurable disease, stage, and performance status within 1 week before starting LMB-2.
- ECG and CXR within 2 weeks before starting LMB-2.
- Echocardiogram and CT scan of the chest, abdomen, and pelvis within 4 weeks before starting LMB-2.
- Serum anti-LMB-2 antibody assay, HIV, Hepatitis B surface antigen, and C screen within 2 months before starting LMB-2.
- Laboratory evaluation within 1 week before starting LMB-2 will include CBC/differential, acute care panel (electrolytes, glucose, BUN, creatinine), hepatic panel (AST, ALT, Alkaline phosphatase, total and direct bilirubin), albumin, LDH, PT, PTT, urinalysis. A 24 urine creatinine clearance and total protein will be done if Cr >2.0
- Urine or serum pregnancy test within 3 days before starting LMB-2 in women with childbearing potential.
- Skin punch biopsy to evaluate tumor infiltration in the skin within 3 months before starting LMB-2.
- If Sezary cells are present in the peripheral blood, a FACS analysis will be done within 1 week before starting LMB-2.
- Bone marrow biopsies will be done on all patients with stage IIa disease and higher within 1 month before starting LMB-2, but may be cancelled at the discretion of the PI.

2.3 PATIENT REGISTRATION

Authorized staff must register with Central Registration (CR) (currently performed via a contract with the Harris Corporation) an eligible candidate within 24 hours of signing the consent. A registration checklist must be completed and faxed to CR at 301-480-0757.

After confirmation of eligibility at CR, CR staff will call the pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Authorized staff must notify CR when a patient is taken off study.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase II study to test the response of patients with cutaneous T cell lymphomas to LMB-2. LMB-2 will be given every 25 to 28 days for up to 6 cycles in the absence of neutralizing antibody production; and up to 3 additional cycles can be given to patients who have a documented ongoing response between cycles 4 and 6. Patients who relapse after >2 months of a CR or PR are eligible for retreatment on the same schedule. Patients will be evaluated according to the eligibility criteria in section 2 and eligible patients will be enrolled on study. Patients will be treated with LMB-2 as described below. Response and duration of response will be determined as described in section 5. If patients consent, biopsies may be obtained from patients with solid lesions, and apheresis may be performed for patients with circulating Sezary cells for molecular analysis prior to and after treatment.

3.1.1 Dose levels

Six patients will be enrolled at a dose level of 30 ug/Kg QODx3, and if 0-1 experience DLT, the dose level may be raised to 40 ug/Kg QOD x3 after consultation witih CTEP.

3.2 DRUG ADMINISTRATION

LMB-2 Infusion: 30 µg/Kg 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). It will be given with IV fluid as described below. The first cycle will be administered on the Oncology Inpatient Unit. If treatment is tolerated well, then subsequent doses can be administered on an outpatient basis.

Premedication: Patients will be premedicated 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. Dexamethasone 20 mg i.v. will be given 2 hours prior the 1st dose of each retreatment cycle. 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 1000 ml of D5 ½ normal saline over 2-4 hours prior to LMB-2 and another 1000 ml of D5 ½ normal saline over 2-4 hours after the LMB-2 infusion is completed. (This is approximately the same amount of IVF given to the last 4 patients on the phase I protocol. However, it is in a more simple administration format). Once the first dose of each cycle is given, patients will receive normal saline, at least 40 cc/hr until day 5-8 when not receiving another type of I.V. fluid.

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 by > 20 mmHg, then another standing blood pressure measurement will be done at least 3 minutes after the first. Please see section 4.0 for management of hypotension. 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. Please see section 4.0 for management of hypotension.

3.3 TREATMENT MODIFICATIONS

3.3.1 Definition of DLT:

- Based on CTC4.0 with a possible (3), probable (4), or definite (5) attribution.
- Grade III-IV toxicities except for:
    - Grade 3 or 4 AST, ALT, GGT lasting ≤ 2 weeks is not dose-limiting.
    - Fever ≥ 38C for 24 hours is not dose limiting.
    - Grade III Blood/Bone marrow toxicity, and Grade IV ANC or platelets lasting <5 days in patients with lymphoma in the bone marrow or peripheral blood.
- Definition of Capillary Leak Syndrome (stipulated by CTEP): As specified by CTC 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 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 CLS will be considered also as 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 considered a grade III CLS. Grade III hypotension or grade III CLS is dose limiting.

3.3.2 Treatment Modifications for Toxicities on Current Cycle

Grades I and II Allergic Reaction/hypersensitivity (including drug fever):

- Drug fever only: give acetaminophen every 4-6 hours until resolved (see section 4.0). Drug will be held (up to 3 days) until fever is <38° C (single measurement).
- Study subjects who experience grade 2 allergic reactions of asymptomatic bronchospasm or generalized urticaria in the face of planned premedication may not be re-treated and must be removed from study.

Grade II Creatinine: Drug will be held (up to 3 days) until Creatinine is ≤ Grade I.

Grade III DLT: Stop treatment. Cycle ends.

Grade IV DLT & Grade III Allergic Reaction: Stop treatment. Off study except for DLT due to grade IV CPK resolving to < grade II within 2 weeks, and not associated with any other DLT.

3.3.3 Treatment Modifications for Toxicities on Previous Cycle

Retreatment:

- Prior to re-treatment, study subjects must have recovered from all toxicities to ≤ grade 1 except for subjects who entered the study with ≥ grade 2 baseline (abnormal) hematological values in which case blood counts should have recovered to baseline values prior to re-treatment.

Dose reduction if at least 1 of the following occurred:

- Grade III DLT or grade IV CPK resolving to < grade II within 2 weeks, not associated with any other DLT.
- 2nd or 3rd dose held >1 day (due to toxicity)
- Grade II creatinine
- Any 2 of these 3:
    - Grade II hypoalbuminemia
    - Grade II weight gain
    - Grade I creatinine

3.3.4 Dose Reductions:

- The 1st dose reduction is by 10 µg/Kg QOD x 3 of the original dose.
- The 2nd dose reduction is by 20 µg/Kg QOD x 3 of the original dose.
- If a 3rd dose reduction is required, patient is off study.

3.3.5 Cycle limitation:

- Subjects may not receive more than two cycles of LMB-2 therapy after achieving a complete response.

3.4 PHARMACOKINETIC STUDIES

3.4.1 Analysis of LMB-2 in the plasma

Blood samples will be drawn by the patient care unit registered 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 dose. Samples drawn from central venous catheters may be used as long as it is flushed with 5 ml of 0.9% sodium chloride prior to obtaining the 2 minute post-infusion sample. Samples of about 3 ml of blood will be drawn in a 6 ml sodium heparin tube (green top). Samples of 2 ml blood in serum separator tubes will be drawn with each greeen top tube to allow ELISA assays to be run on stored serum samples. Tubes of blood should be stored upright in the

"Kreitman" container in the refrigerator in the soiled utility room. These samples will be collected daily Monday through Friday and taken to our lab for analysis.

3.4.1.1 Day 1

Inpatients: Pre-dose, 2 minutes post (after end of 30 minute infusion), then at 1, 2, 3, 4, and 12-24 hours post.

Outpatients: Pre-dose, 2 minutes post (after end of 30 minute infusion), then at 1, 2 hours and 12-24 hours post.

3.4.1.2 Day 3

Pre-dose, 2 minutes post, then 12-24 hours post.

3.4.1.3 Day 5

Pre-dose, 2 minutes post, 1 hour post, then 12-24 hours post.

The acceptable error for PK time points is +/- 2 minutes for 2 minute sample; +/- 15 minutes for other samples. The actual time of sample collection should be recorded.

3.4.2 Analysis of neutralizing antibodies & soluble Tac

At least 2 ml of blood will be drawn in a Serum Separator Tube (SST) prior to starting each cycle and between days 17-25 of each cycle. Tubes must be labeled with the patient's name, medical record number, date of birth, date, and time drawn. Tubes of blood collected at the clinical center should be stored upright in the "Kreitman" container in the refrigerator in the soiled utility room. These samples will be collected daily Monday through Friday and taken to our lab for analysis. Tubes of blood collected outside of the clinical center should be sent by Fed Ex to: David Waters, Ph.D. — SAIC: Building 560, lab 11/09, 1050 Boyles St. Frederick, MD 21702 — Phone: 301-846-5831.

The presence of antibodies to LMB-2 is determined by a bioassay performed in a CLIA-certified lab in Frederick. In a 96-well U-bottom plate, serum from patients, or 0.2% human serum albumin in PBS (HSA-PBS), is mixed with different concentrations of LMB-2 which are diluted in HSA-PBS. After mixing with serum, the serum-toxin mixtures each contain 90% serum and either 0, 40, 200 or 1000 ng/ml of LMB-2. These mixtures are incubated at 37C for 15 minutes and diluted into cell culture media (DMEM + 10% FBS) in a U-bottom 96-well plate so that the final toxin concentrations are 0, 0.16, 0.8, or 4 ng/ml. In triplicate, 50 ul aliquots of these diluted serum-toxin mixtures are added to 150 ul aliquots of SP2-Tac cells (40,000/well in DMEM + 10% FBS) in 96-well flat-bottom plates. After incubating the cells for 16-20 hours at 37C, the cells are pulsed for 4-5 hours with [^{3}H]-leucine, harvested, and counted to determine inhibition of protein synthesis. Percent neutralization is calculated by determining the % inhibition of protein synthesis of toxin in HSA-PBS, minus the % inhibition in serum, divided by the % inhibition in HSA-PBS, multiplied by 100. For example, if the 1000 ng/ml concentration of LMB-2 + HSA-PBS caused 75% inhibition of SP2/Tac cells and this concentration in serum caused 50% inhibition, the % neutralization would be 33%.

3.4.3 Analysis of additional research blood

Other blood samples (≤25 ml per 4 weeks) may be drawn for research purposes. There will be no genetic or germ line testing unless the patient is reconsented.

3.5 PROTOCOL EVALUATION

3.5.1 Routine Tests done during Each Cycle

3.5.1.1 On days 2-8

- CBC/diff, chem 20, CRP and urinalysis.

3.5.1.2 Once on day 7 or 8

- GGT, IgG, IgA, IgM, prealbumin, PT, fibrinogen, amylase, lipase, haptoglobin,

3.5.1.3 Once between days 17-25

- Neutralizing antibody, CBC/diff, creatinine.

3.5.2 Prior to Each Subsequent Cycle the following will be done:

- Restaging will be completed prior to each cycle. This will include an interim history and physical examination with documentation of measurable disease, stage, and performance status.
- A skin punch biopsy will be done prior to cycle 2 and may be done prior to subsequent cycles if clinically relevant.
- If Sezary cells are present in the peripheral blood, a FACS analysis will be done.
- Laboratory evaluation within 3 days will include CBC/differential, acute care and hepatic panels, albumin, LDH, PT, PTT, urinalysis.
- ECG, CXR.

3.6 CONCURRENT THERAPIES-SEE SECTION 4.0

3.7 SURGICAL & RADIATION THERAPY GUIDELINES-NOT APPLICABLE

3.8 OFF TREATMENT CRITERIA

- Progression of disease during active treatment on study protocol.
- Grade III Allergic Reaction & Grade IV DLT as defined in section 3.3.
- More than 2 dose reductions are required.
- Patient non-compliance or voluntary withdrawal.
- Development of neutralizing antibodies.

3.9 POST TREATMENT EVALUATION (OFF TREATMENT BUT ON STUDY)

Patients will be taken off treatment when they meet off treatment criteria. Patients who are off treatment due to toxicity will be followed until resolution of their side effects. On- or off-study patients who have not had progressive disease will be followed every 3-12 months until they have progressive disease. Patients who have progressive disease may also need follow-up for other endpoints, including neutralizing antibodies. Post-

treatment data including neutralizing antibody levels may be obtained from clinic visits either at NIH or elsewhere.

3.10 OFF-STUDY CRITERIA

- Patient begins different therapy
- Unwillingness to continue follow-up
- No further data collected.

4 SUPPORTIVE CARE

- Allergic reaction will be treated acutely with antihistamines (including diphenhydramine, hydroxyzine, & ranitidine), fluids, bronchodilators, and/or epinephrine.
- Nausea and Vomiting: Patients who develop nausea will be treated with a serotonin 5-HT 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.
- 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 NSAIDs or narcotics if acetaminophen is inadequate.
- Capillary leak syndrome: supportive care may include fluid and electrolyte management, diuresis, albumin, and cardiovascular support.
- Hypotension: Patient will be encouraged to increase oral fluid intake. In addition, for an orthostatic SBP change of >20, an IVF bolus may be given. Refractory hypotension may require treatment in the intensive care unit with pressors.
- Fever: Patients who develop temperatures >38.0 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.
- 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. 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/mm3.
- 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.
- Febrile Neutropenia is a 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.
- 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.
- 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. Given that this type of cancer can cause profound difficulty in the areas of potential disfigurement and threat to self-esteem, Social Work will be proactively involved with these patients' biopsychosocial well-being.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

5.1.1 Procedures

This study will be monitored by CTMS. For data safety and monitoring, the Principle Investigator, Dr. Robert Kreitman, will monitor all adverse events. Unexpected adverse events and/or serious adverse events will be reported to the NCI IRB and the study sponsor as described in section 7.0. If trends are noted and/or risks warrant it, accrual will be interrupted and/or the protocol and/or consent document will be amended accordingly.

5.1.2 Study Monitor: CTEP

5.2 RESPONSE CRITERIA

There are no published guidelines for CTCL skin tumor burden assessments (78). Their validity is grounded in their reproducibility, ease of assessment, accuracy, and objectivity. The denileukin and bexarotene trials developed different, complex, and time-consuming objective and subjective methods for quantifying tumor burden in the skin. The FDA based approval of these drugs only on the objective measurements of tumor burden (1,7,15,77).

Both of these objective measurement schemes multiplied the area of skin involved by a factor weighted for the type of lesion. The denileukin diftitox trial used a factor of 1 for a patch, a 2 for a plaque, and a 4 for a tumor (erythema was not measured but given a subjective score by the investigator). In patients with ≤ 10% of BSA involved, 5 target skin lesions were measured (preferably one of each type). For patients with >10% BSA involvement, a standardized grid was used to map and calculate the total BSA involved. The Bexarotene trial also chose 5 target skin lesions to follow. Their Composite Assessment Grading Scale (CA) rated 4 characteristics (erythema, scaling, plaque elevation, pigmentation) on a 9 point scale and BSA on an 18 point scale and used a complicated formula to determine a final score. In both trials non-target skin lesions were followed for CR or PD.

Response criteria for this trial will be based on the International Workshop's Response Criteria (IWRC) for Non-Hodgkin's Lymphomas (75) which favors the sum of the bidimentional products for tumor measurements. Skin tumor burden assessment will also incorporate the use of 5 target lesions and weighted multiplication factors based on the trials noted above. The denileukin trial considered a ≥50% reduction of Sezary cells by FACS analysis as a PR in that category so we will also use that criteria.

5.2.1 Evaluation of Response:

Skin, lymph node, Sezary cells, and bone marrow will be evaluated for response. Disease that will be measured includes up to 5 target skin lesions, up to 5 lymph nodes, and Sezary cells. All measured skin lesions and lymph nodes will be identified prior to starting cycle 1 and will be followed prospectively. Non-target skin lesions and non-target lymph nodes will be followed for CR or PD. Non-measurable disease includes

diffuse erythroderma and bone marrow. CTCL measurement worksheets (such as the one in the appendix) will be used to document tumor burden. Photographed skin lesions will be identified prior to cycle 1. Photography will then be done prior to starting each cycle. Patient consent for photography will be obtained prior to enrolling on the study.

- Complete Response (CR): no evidence of disease—CR in every category.
- CR/unconfirmed (CRu): CR in every category except CRu in lymph nodes.
- Partial response (PR): PR in every measurable disease category with non-progressive disease elsewhere.
- Progressive Disease (PD): PD in any category.
- Stable Disease (SD): Neither PR nor PD.

5.2.2 Evaluation of Measurable Disease

5.2.2.1 Measurable Skin Lesions

In patients with measurable skin lesions, up to five representative target lesions will be chosen at baseline. They will be measured at the beginning of each cycle and at each follow-up visit. All other skin lesions will be considered non-target lesions. The Target Skin Score will be calculated and recorded using the CTCL TARGET SKIN LESION SCORING INDEX in the appendix.

- Complete Response (CR): Disappearance of all skin lesions
- Partial Response (PR): ≥ 50% reduction in Target Skin Score, and non-progressive non-target lesions.
- Progressive Disease (PD): ≥ 50% increase in Target Skin Score, appearance of new lesions, or unequivocal progression of existing non-target lesions.
- Stable Disease (SD): Neither PR nor PD in Target Skin Score and/or persistence of one or more non-target lesions.

5.2.2.2 Lymph Nodes

In patients with lymph node involvement (N1), up to five lymph nodes (or lymph node masses) will be chosen at baseline and considered target lymph nodes. They will be measured at the beginning of each cycle and at each follow-up visit. All other lymph nodes (or lymph node masses) will be considered non-target lymph nodes. Lymph node measurements based on the CT scan will be recorded at the beginning of each cycle. A sample lymph node record sheet is in the appendix.

- Complete Response (CR): All lymph nodes must have regressed to normal size; lymph nodes >1.5 cm before therapy must decrease to ≤1.5 cm; lymph nodes 1.1-1.5 cm prior to therapy must decrease to ≤1 cm.
- CR/unconfirmed (CRu): Patients with a residual lymph node greater than 1.5 cm that has regressed by more than 75% in the sum of the products of the greatest perpendicular diameters (SPD) of the target lymph nodes. Individual nodes that were

previously confluent must have regressed by more than 75% in their SPD compared with the size of the original mass.

- Partial response (PR): ≥50% decrease in SPD of the five largest target nodes or nodal masses.
- Progressive Disease (PD): At least a 50% increase from nadir in the SPD of any previously identified abnormal node for PRs or nonresponders, or the appearance of any new lesion during or at the end of therapy.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

5.2.2.3 Sezary Cells

In patients with peripheral blood Sezary cells, a FACS will be done prior to each cycle and recorded (sample record sheet is in the appendix).

- Complete Remission: Disappearance of all Sezary cells (<5% of the lymphocytes by FACS) for a period of at least 4 weeks.
- Partial Response (PR): ≥50% reduction of Sezary cells sustained for at least 4 weeks.
- Stable Disease: neither PR nor PD.
- Progressive Disease (PD): ≥50% increase of Sezary cells (>5% of the lymphocytes) from pre-treatment levels.

5.2.3 Evaluation of Non-measurable disease

5.2.3.1 Diffuse Erythroderma

Because diffuse erythroderma measurement requires a subjective evaluation by the investigator, it will only be used to determine if a patient has a CR. Erythroderma severity will be recorded prior to each cycle in applicable patients.

- Complete Response (CR): Disappearance of all erythema.
- Stable Disease (SD): Persistence of erythroderma.
- Progressive Disease (PD): Appearance of erythroderma after >4 weeks of CR within 60 days of their last dose.

5.2.3.2 Bone Marrow

In patients with bone marrow involvement, a bone marrow biopsy will be done if clinically indicated prior to each cycle (sample record sheet is in the appendix).

- Complete Response (CR): No evidence of lymphoma by histology or immunohistochemistry.
- Partial Response (PR): irrelevant because bone marrow is assessable and not measurable disease.

- Progressive Disease (PD): Reappearance of lymphoma in the bone marrow after a documented negative biopsy.

5.2.4 Confirmation of Response & Duration of Response

5.2.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met SD criteria at least once after study entry at a minimum interval of 4 weeks.

5.2.4.2 Duration of Overall Response

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

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression, even after discontinuation of treatment.

5.2.4.3 Duration of Stable Disease

Stable disease is measured from the start of treatment until the criteria for progression are met. In this study, we will consider stable disease lasting at least 4 months as significant.

5.3 TOXICITY CRITERIA

5.3.1 CTC version

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 July 31, 2010 for AE reporting. CTCAE version 4.0 will be utilized beginning August 1, 2010.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).

5.4 STATISTICAL SECTION

5.4.1 Race, Ethnicity, and Gender

Enrollment will be extended to individuals of all racial/ethnic categories to both genders. Statistical analysis to explore ethnic, race, or gender differences will not be performed, as this study is not powered to investigate these differences.

5.4.2 Accrual

The primary objective of this trial will be to determine if LMB-2 can produce clinical responses in a reasonable proportion of patients, and thus warrant further testing. The trial will be conducted using a two-stage Minimax design (76). With alpha = 0.10 (the probability of incorrectly accepting a poor agent), and beta = 0.10 (the probability of incorrectly rejecting a good agent), we will try to rule out an undesirably low response probability of 10% (p0=0.10) in favor of a level indicative of acceptable activity, 30% (p1=0.30).

Initially 16 evaluable patients will be enrolled. If 0-1/16 demonstrate an objective response (per section 5.2), then accrual will stop and the agent will be considered inactive in this population. If 2+/16 patients have a response, accrual will continue until a total of 25 patients have been enrolled. If 2-4 of 25 respond, this will be considered insufficient activity, and the agent will not be considered worthy of further development. If 5+/25 respond, then the agent will be considered active (and at least potentially able to produce a response level consistent with 30%). Under the null hypothesis (p0=0.10), the probability early termination of this trial is 0.51.

Duration of response is also important to evaluate in this population, since responses of greater than 4 months duration are not common but would be considered desirable. A statistical summary of the duration of response will be reported, along with the fraction of responding patients who achieve a four month response duration, and the corresponding 95% confidence interval. In addition, a Kaplan-Meier curve of response duration will be created to illustrate the pattern associated with this outcome.

Because CTCL is a rare disease, patient accrual will not be stratified by stage of disease, but exploratory evaluations will be performed after all patients have been enrolled and evaluated to determine whether there is a relationship between response obtained and stage of disease.

Secondary objectives of the trial include describing the relationship between immunogenicity, toxicity, and serum concentrations of LMB-2, as well as to determine if soluble Tac-peptide (sIL2R alpha) levels correlate with response to treatment with LMB-2. These 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. 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.

It is expected that 1-2 patients per month can be recruited for enrollment onto this trial, and with a goal of 25 evaluable patients, it is expected that 2 years is a reasonable time frame in which to accrue all needed subjects. In the event that a small fraction of patients are not evaluable for response, up to 2 extra (total of 27) will be allowed to be enrolled onto this trial.

6 HUMAN SUBJECTS PROTECTIONS

6.1 RATIONALE FOR SUBJECT SELECTION

6.1.1 Selection based on gender, ethnic background or race

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.

6.1.2 Strategies/Procedures for Recruitment

Referrals from within the NCI and National Naval Medical Center are expected, but we anticipate that most of the referrals will come from physicians outside of our institution. Letters describing the protocol may be mailed, emailed, or faxed to inquiring and other potential referring physicians. This protocol will also be available through the PDQ database and the Bethesda Trials Hotline number 888-624-1937.

6.1.3 Justification for Exclusions

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 B antigen positivity, hepatitis C PCR positivity, or other chronic liver disease are excluded from this trial because the effect of LMB-2 on hepatitis B and/or the immune system is unknown and potentially harmful. Patients that are pregnant or breast-feeding will be excluded from this trial because the effect of LMB-2 on the developing fetus or the nursing infant is unknown and potentially harmful.

6.2 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.

6.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

6.3.1 Potential benefits to subjects expected from the trial

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.

6.3.2 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.

6.3.3 Procedures for protecting against or minimizing any potential risk

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 improve 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.

6.3.4 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 to register and verify patients' eligibility.

6.4 Risks/Benefits Analysis

Patients enrolled on this study will be those with a disease that is considered incurable. They will generally have a poor prognosis and have no standard treatment options known to significantly improve survival. Thus, patients may experience significant treatment related morbidity, and/or have progressive complications of their disease. Although LMB-2 is an experimental new immunotoxin, another drug with a similar mechanism of action and similar toxicities was recently approved for CTCL. In addition, we have seen responses in patients with CTCL to LMB-2 with acceptable toxicities. Consequently, we believe that patients participating in this trial will have an acceptable benefit/risk ratio.

6.5 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. Telephone consent may be employed in order to screen outside samples from prospective subjects for CD25 expression and expression of neutralizing antibodies. In such cases, a protocol investigator will review the Screening Sample Consent form by telephone. The consent/assent signatures will be witnessed by an observer present with the patient and a copy will be faxed and the original sent by mail to the PI. Prospective subjects who consent to send such samples for outside testing will NOT be registered with the NCI Central Registration Office unless they are subsequently enrolled on protocol. Subjects and their referring medical team will be notified of the results and records will be maintained with the protocol research files.

6.6 STORING SPECIMENS

6.6.1 Description of data/specimens:

Blood, bone marrow, lymph node, skin, and other tumor samples. Examples of tests thay may be saved:

- Neutralizing antibodies: Antibodies a patient might make which block the effect of certain recombinant immunotoxins like LMB-2. Requires about 1 teaspoon of blood.
- Flow cytometry assays to quantify tumor markers on the malignant cells. Requires about 1/2 teaspoon of blood.
- Bone marrow biopsy samples, whether they obtained at NIH or elsewhere, and whether the bone marrow test has already been done or not yet done.
- Cytotoxicity assays. Cutaneous T-cell 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 of blood.
- 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 of blood.
- HLA typing to better understand the immune system in patients with Cutaneous T-Cell Lymphoma. Requires about 1 teaspoon of blood.
- PAX-gene tube: To obtain RNA to study the mechanism of how Cutaneous T-Cell Lymphoma cells form, and to detect very low levels of

cutaneous T-Cell lymphoma cells in patients. Requires about 1/2 teaspoon of blood.

- RNA samples can also be used, in an assay called micro-arrays, to study why some patients may not respond as well as others to recombinant immunotoxins like LMB-2. Taken with PaxGene tube.
- Assays which could have an impact on both patients and their children, including studies of genetic cancer risk, will not be done.
- Samples to determine levels of immunotoxin in blood, urine, and other tissues.

6.6.2 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.

6.6.3 Timeframe and location of storage:

Samples will be stored and cataloged longer than a year, in alarmed freezers at our SAIC contract lab in Frederick, MD where neutralizing antibodies and PK samples are tested. The contact information is: David Waters, PhD, SAIC Building 560, Lab 11-09, 1050 Boyles St, Frederick, MD 21702, Phone: 301-846-5831. Portions of samples which are stored at SAIC Frederick 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 SAIC Frederick 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.

6.6.4 Confidentiality:

Patient names or identifiers will not be used in publications resulting from testing of patient samples. Samples shipped to other locations 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.

7 DATA REPORTING

7.1 ADVERSE DRUG REACTIONS

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 & 52	Grades 4 & 52
	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:

AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

AdEERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

2 Although an AdEERS 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 must be reported using expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

Comprehensive Adverse Events and Potential Risks List (CAEPR)
for
LMB-2 Immunotoxin (NSC #676422)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single, complete 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 Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_adeers 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 Immunotoxin.

Version 1.2, February 3, 2010[1]

Adverse Events with Possible Relationship to LMB-2 Immunotoxin (CTCAE 4.0 Term)	**EXPECTED AEs FOR ADEERS REPORTING** **Agent Specific Adverse Event List (ASAEL)**
CARDIAC DISORDERS	***Expected***

	Left ventricular systolic dysfunction	***Left ventricular systolic dysfunction***
	Restrictive cardiomyopathy	
GASTROINTESTINAL DISORDERS		
	Diarrhea	
	Nausea	***Nausea***
	Vomiting	***Vomiting***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
	Edema limbs	
	Fatigue	
	Fever	***Fever***
IMMUNE SYSTEM DISORDERS		
	Allergic reaction	***Allergic reaction***
INVESTIGATIONS		
	Alanine aminotransferase increased	***Alanine aminotransferase increased***
	Alkaline phosphatase increased	
	Aspartate aminotransferase increased	***Aspartate aminotransferase increased***
	CPK increased	
	Creatinine increased	***Creatinine increased***
	Platelet count decreased	***Platelet count decreased***
	Weight gain	
METABOLISM AND NUTRITION DISORDERS		
	Hypoalbuminemia	***Hypoalbuminemia***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Muscle weakness[2]	
	Musculoskeletal and connective tissue disorder - Other (acute rhabdomyolysis)	
	Myalgia	
RENAL AND URINARY DISORDERS		
	Hematuria	***Hematuria***
	Proteinuria	***Proteinuria***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
	Dyspnea	
VASCULAR DISORDERS		
	Capillary leak syndrome	
	Hypotension	***Hypotension***

[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]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.

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

Also reported on LMB-2 Immunotoxin trials but with the relationship to LMB-2 Immunotoxin still undetermined:
CARDIAC DISORDERS - Myocardial infarction; Pericardial effusion; Supraventricular tachycardia
INFECTIONS AND INFESTATIONS – Infection[3]
INVESTIGATIONS - Cardiac troponin I increased

Animal Data: The following toxicities have been observed in animal studies with LMB-2 Immunotoxin: leukocytosis; anorexia

Note: LMB-2 Immunotoxin 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.

- 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..
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS 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 AdEERS.
- In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into AdEERS by the original submitter at the site.
- All AEs reported via AdEERS must also be reported via the routine AEs reporting defined by the protocol.

7.2 EXPECTED ADVERSE EVENTS

7.2.1 Grade 4: **none**

7.2.2 Grade 3: AST, ALT, GGT, hypoalbuminemia, thrombocytopenia.

7.2.3 Grade 2:

- Blood Bone Marrow: thrombocytopenia, hemoglobin, ANC, WBC, lymphopenia.
- Cardiovascular: Capillary Leak syndrome (CTEP defined section 3.3.1), edema, hypotension, pericardial effusion/pericarditis, PTT, PT.
- Constitutional: fatigue, fever, weight gain.
- GI: nausea, vomiting, diarrhea.

- Hepatic: hypoalbuminemia, AST, ALT, GGT, alkaline phosphatase.
- Metabolic/Laboratory: bicarbonate, CPK, hypocalcemia, hypokalemia, hypomagnesemia, hyponatremia, hypophosphatemia.
- Musculoskeletal: muscle weakness.
- Pain: myalgia.
- Renal: creatinine, proteinuria.

7.3 NCI-IRB ADVERSE EVENT REPORTING REQUIREMENTS:

7.3.1 Definitions:

7.3.1.1 Suspected adverse reaction

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

7.3.1.2 Unexpected adverse reaction

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

7.3.1.3 Serious

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

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.3.1.4 Disability

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

7.3.1.5 Life-threatening adverse drug experience

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

7.3.1.6 Protocol Deviation (NIH Definition)

A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has not been approved by the IRB.

7.3.1.7 Protocol Violation (NIH Definition)

Any change, divergence, or departure from the study procedures in an IRB-approved research protocol that has a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

7.3.1.8 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**

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

7.3.2 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

The protocol PI will report to the NCI-IRB:

- All unexpected serious adverse events that are possibly, probably, or definitely related to the research
- All deaths, except deaths due to progressive disease

- All Protocol Violations or Deviations
- All Unanticipated Problems

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

7.3.3 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

The protocol PI will report to the NCI-IRB:

1. All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
2. All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
3. All Grade 5 events regardless of attribution;
4. All Serious Events regardless of attribution.

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

7.3.4 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that require a sponsor recommended change to the protocol or the consent form or in the opinion of the PI increases risks to study participants will need to be reported to the NCI IRB.

7.4 RECORD KEEPING

- Complete records must be maintained on each patient; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, 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.
- An electronic research record including the following items will be kept on NetTrials or other MOCRU/CCR approved database: 1. On/off study dates, 2. response and progression dates, 3. drug administration with dose and cycle, 4. toxicity with grade and attribution, 5. concomitant medications.
- 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.
- Data will be submitted to CTEP every two weeks. The data will be submitted electronically.

7.5 SECONDARY MALIGNANCY

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via AdEERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 SECOND MALIGNANCY

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

7.7 DATA AND SAFETY MONITORING PLAN

- The research nurse will ensure that data, reporting, and adverse events will be reviewed at least every other week. Unexpected events will be monitored for trends. Amendments to the protocols and consents will be made to protect the patients and answer important scientific questions that arise.
- Intramural quality assurance monitors will be monitoring the protocols yearly.
- 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.

8 PHARMACEUTICAL INFORMATION

8.1 LMB-2 IS AN INVESTIGATIONAL RECOMBINANT IMMUNOTOXIN.

- The NSC number for LMB-2 is 676422. CTEP, DCTD is the sponsor of the IND (IND # 6662). Anti-Tac(Fv)-PE38 (LMB-2) is classified as a recombinant immunotoxin containing 589 amino acids and has a molecular weight of 63 kDa.

- The manufacturer of the bulk product is The Monoclonal Antibody and Recombinant Protein Facility (MARP) of the NCI in Frederick, MD. All batches of the product were vialed at the MARP except for the 1st batch which was vialed at the NIH Clinical Center Pharmacy.
- How supplied: Vials will be supplied by CTEP, NCI and may be ordered from the Pharmaceutical Management Branch via usual procedures. Each mL contains LMB-2 in phosphate buffered saline. The strength of the immunotoxin varies with each lot and is specified on the label of the individual vial. The pH is approximately 7.4.
    - The strength of the 1st batch was 420 µg/mL of LMB-2. This is referred to as Lot #64956. It was used until November 1997.
    - The strength of the 2nd batch was 534 µg/mL of LMB-2 (1 mL of a sterile frozen solution in a clear 2 mL vial). This is referred to as Lot # 32606. This batch was used from February 1998 until January 1999.
    - The strength of the 3rd batch is 436 µg/mL of LMB-2 (2 mL of a sterile frozen solution in a 10 mL vial). This is referred to as Lot # 103037.(No LMB-2 was used from 1/99—3/01).
    - The strength of the 4th batch is 484 mcg/mL of LMB-2 (2 mL of a sterile frozen solution in a 10 mL vial). This is referred to as Lot # 103038.
- Comparability & Displacement Assays have been done frequently comparing the different lots to each other and to the standard lot (Lot #A). In the Comparability assays, SP2/Tac or HUT-102 cells were incubated with a sample of each lot for 15-20 hours at 37°C, pulsed with [^{3}H]leucine for 4-6 hours, harvested, and counted. The IC_{50} is the concentration of LMB-2 which inhibits protein synthesis by 50%. In the Displacement assays SP2/Tac cells were incubated with 0.1 nM [^{125}I]-humanized anti-Tac (HAT) in the presence or absence of increasing concentrations of LMB-2. After 1-2 hours of incubation, the cells were washed by centrifugation and counted to determine the amount of [^{125}I]-HAT bound. The EC_{50} is the concentration that displaces radiolabeled anti-tac by 50%. The activity of these lots in both assays are not significantly different. Specific data can be provided upon request. Comparability and displacement assays will continue to be done on a regular basis. The table below lists the dates various lots were tested.

Date compared	**Lot Numbers compared**				
	A	64956 (1st batch)	32606 (2nd batch)	103037 (3rd batch)	103038 (4th batch)
1/13/98	X		X		
1/30/98	X	X	X		
6/10/98	X		X		
3/20/00	X			X	
4/18/00	X			X	X
9/19-21/00		X	X	X	X
1/9/01, 4/4/01				X	X

4/16/01			X	X	X
5/1-8/01			X	X	X
9/11/01			X	X	X

- Prior to each lot release every lot underwent size exclusion chromatography to determine the percentage of aggregates in the sample.
- Preparation:
    - Thawing: Vials should be warmed in the hand for 10-20 seconds before they are placed into a water bath to thaw. Vials will be placed in a cup of room temperature (15-30°C) Sterile Water for Irrigation, USP (SWI) so that when the vial is standing upright, the water level will be at the neck of the vial. Vials should be visually inspected after thawing. Do not use if material appears turbid.
    - **"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."**
    - **Please refer to the administration section below for additional preparation information.**
- Storage: Store the intact vials in the freezer (-70°C or below). The IV admixture should be stored in the refrigerator (2°C-8°C). ***Vials should not be thawed and refrozen***.
- Stability: Intact vials of LMB-2 are felt to be stable for at least 5 years when stored at –70°C. Stability of the intact vials, once thawed, is at least 24 hours when stored in the refrigerator (2°C-8°C), and for 4 hours when stored at room temperature (15-30°C). The LMB-2 admixture, 14µg/mL, in 0.9% Sodium Chloride Injection, USP with HSA 0.2% yields a solution that is stable for 25 hours; 1 hour in the IV bag stored at 25°C, and 24 hours of infusion time. Once thawed, the vials should not be placed back in the freezer (for future prescriptions), as they are not stable to freeze-thaw conditions.
- Route of Administration: Intravenous (IV).
- Administration: A test dose of LMB-2 will not be given on this protocol. Prehydration, premedication, and LMB-2 administration procedures are detailed in section 3.2. To prepare LMB-2, vials will be thawed and the required volume of LMB-2 will be qs'd to 50 mL in a PAB containing 0.9% Sodium Chloride Injection, USP with 100mg HSA such that the final albumin concentration is 0.2%. 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. Treatment doses will be administered as an IV infusion over 30 minutes. After administration of the treatment dose, the line will be

flushed with 0.9% Sodium Chloride Injection, USP. A PAB (Partial Additive Bag) 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 the 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 (i.e. paclitaxel). The NIH Clinical Center uses this 150 ml capacity PAB Mixing Container when preparing LMB-2 because it is the standard empty sterile container available.

- Compatibility: LMB-2 should only be mixed in 0.9% Sodium Chloride Injections, USP with HSA 0.2%. There are no known drug interactions.
- Special Handling: LMB-2 should be handled and labeled as a hazardous drug.

8.2 TOXICITY

8.2.1 Preclinical studies

In a GLP toxicology study, 4 Cynomolgus monkeys received 20 µg/Kg days 1, 3 and 5 with no significant toxicity. Another four monkeys were then given 300 µg/Kg 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 µg/Kg every other day for 3 doses. The cause of death was liver damage.

8.2.2 Phase I trial

Grade III-IV toxicities included reversible transaminase elevation (8), fever (2), CK elevation (1), cardiomyopathy (1), thrombocytopenia (1), allergic reaction (1), and diarrhea (1).

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.

8.3 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.

8.3.1 Acetaminophen (Tylenol):

- Side effects are extremely unlikely. Regular use of acetaminophen can cause liver damage especially at high doses (above 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.

8.3.2 Ranitidine (Zantac):

- Side effects include tiredness, dizziness, headache, and diarrhea.

8.3.3 Hydroxyzine (Atarax):

- Side effects include sleepiness, dizziness, restlessness, and irritability.

8.3.4 Dexamethasone (Decadron):

- To be given only prior to the 1st dose of retreatment cycles. Side effects of a single dose of dexamethasone might include difficulty sleeping, increased hunger, and increased blood glucose. Steroids are associated with an increased risk of infection although this is more applicable to chronic use.

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10 APPENDICES

A: Protocol Flowsheet

B: CTCL Target Skin Lesion Scoring Index

C: Target skin lesion response

D: LMB-2 Neutralization Protocol Using SP2/Tac Cells

E: LMB-2 PK Assay for SP2/Tac Cells

10.1 APPENDIX A: PROTOCOL FLOWSHEET

PHASE II TRIAL OF LMB-2 IN CD25+ CUTANEOUS T CELL LYMPHOMAS										
Protocol # 04-C-0142										
Use this flowsheet with each cycle	**Pre-cycle**	**d1**	**d2**	**d3**	**d4**	**d5**	**d6**	**W2**	**W3**	**W4**
Drug administration		X		X		X				
History (include PS) & Physical exam	X									
Labs:										
CBC/diff & acute care panel	X	X	X	X	X	X	X	X	X	X
Albumin & hepatic panel	X	X	X	X	X	X	X	X	X	X
LDH, PT, PTT, Uric acid, urinalysis	X	X		X		X				
24 hour Urine for CrCl (if serum Cr >2.0)	X									
Pharmacokinetic Studies ~3 ml blood in a 6 ml sodium heparin green top tube + 2ml blood in 3 ml serum separator tube										
Day 1: pre, 2 min, & 1, 2, 3, 4, 12-24 hours		X								
Day 3: pre, 2 min, 12-24 hours				X						
Day 5: pre, 2 min, 1 hour, 12-24 hours						X				
Neutralizing antibodies and Soluble Tac:										
1 Serum Separator Tube	X								X- day 17-25	
Additional tests Each Cycle										
CXR, ECG	X									
Skin tumor measurement & photos	X									
Skin punch biopsy (cycles 1 & 2)	X									
Additional Research labs: optional										
Tests Done prior Cycle 1 (On-study tests)										
CT scan of Chest/Abd/Pelvis	X	No IV contrast within 72 hours of LMB-2 dose								
Echocardiogram	X									
HIV, Hep BsAg & C	X									
Pregnancy test if applicable	X									
Bone Marrow Biopsy if stage II-IV	X									
Tests done prior to each cycle if clinically indicated										
FACS (green-top tube)- if +Sezary cells	X									
Bone Marrow Biopsy	X									
CT scan of Chest/Abd/Pelvis	X									
Echocardiogram	X									

10.2 APPENDIX B: CTCL TARGET SKIN LESION SCORING INDEX

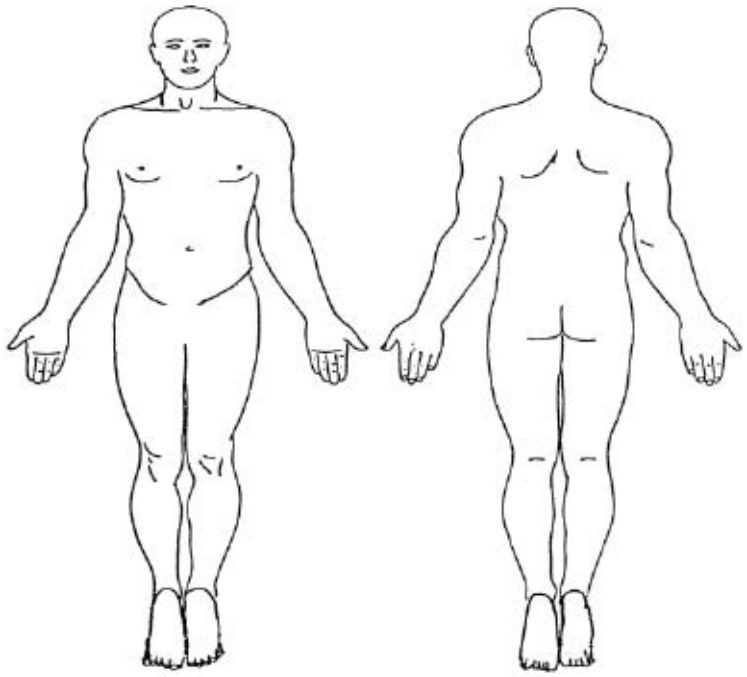
(see section 5.2.2.1)

Name:_____
Protocol:_____
Cycle #:_____

A. Plot on above pictures location of representative lesions and label 1-5.
B. Score representative lesions as they correspond to location number:

LOCATION NUMBER	SIZE IN CM^2	TYPE OF LESION PATCH (1)	PLAQUE (2)	TUMOR (4)	WEIGHTED RESULTS
1._____	____x____ = ____cm^2	O	O	O	_____
2._____	____x____ = ____cm^2	O	O	O	_____
3._____	____x____ = ____cm^2	O	O	O	_____
4._____	____x____ = ____cm^2	O	O	O	_____
5._____	____x____ = ____cm^2	O	O	O	_____

TARGET SKIN SCORE = SUM OF WEIGHTED SCORES:_____



10.3 APPENDIX C: TARGET SKIN LESION RESPONSE

(Up to 5 representative lesions, see section 5.2.2.1)

→Place score and response on Summary Sheet

NA = not applicable

CR = 100% clearing of target lesions
PR = ≥ 50% reduction in Target Skin Score
SD = not PR or PD
PD = ≥ 50% increase in Target Skin Score

NON-TARGET SKIN LESION EVALUATION

(All non-target lesions, see section 5.2.3.1)

→Place score and response on Summary Sheet

Score	Response
0= No Non-Target lesions	NA = not applicable
1= Non-Target lesions present	CR = 0 (PR = NA)
2= Non-Target lesions increased significantly or appearance of new Non-target lesions	SD = did not reach 0
	PD = score increased

ERYTHRODERMA EVALUATION

(Erythema is defined as diffuse and may have associated edema, see section 5.2.3.1)

→Place score and response on Summary Sheet

Score	Response
0= No erythema	NA = not applicable
1= 1-49% of skin involved	CR = 0 (PR = NA)
2= 50-99% of skin involved	SD = did not reach 0
3= Diffuse erythroderma involving 100% of skin	PD = increased from 0

10.4 APPENDIX D: LMB-2 NEUTRALIZATION PROTOCOL USING SP2/TAC CELLS

1. Set up SP2/Tac cell plates using SP2/Tac cell line protocols. Place cell plates in 37°C incubator until needed.

2. Using a costar #3799 U-bottom plate, add 72 ul of .2% PBS/HSA to wells A1-A4 and 60 ul to wells A9-A-12. Add patient serum to the plate in the following manner:

    a. Patient 1: 45 ul of serum in wells D1-D4
    b. Patient 2: 45 ul of serum in wells E1-E4
    c. Patient 3: 45 ul of serum in wells F1-F4
    d. Patient 4: 45 ul of serum in wells G1-G4
    e. Patient 5: 45 ul of serum in wells H1-H4

3. Thaw LMB-2 toxin in hand and add 15 ul to well A12. Mix well. Transfer 15 ul from well A12 to well A11. Mix well. Transfer 15 ul from well A11 to well A10. Mix well. Well A9 does not receive toxin.

4. Using a multichannel pipette, transfer 8 ul from wells A9-A12 into wells A1-A4. Well A1 receives from well A9, well A2 from well A10, etc.

5. In a similar manner, transfer 5 ul from wells A9-A12 into the patient serum wells. For example, well D1 receives from well A9, etc. Dispel pipette tips between additions to patients samples.

6. Using a multichannel pipette, mix wells A1-A4, and all patient sample wells in which toxin additions were made. Dispel pipette tips between each set.

7. Incubate the U-bottom plate at 37°C for 15 minutes.

8. During incubation, prepare two new Costar #3799 U-bottom plates with 200 uL of room temperature SP2/Tac cell media in all used rows.

9. Add 10 uL of .2% PBS/HSA to row C of U-bottom plate #2.

10. When the 15 minute incubation of plate one is complete, add from plate one to plate two in the following manner:

    Using a single channel pipette:
    Transfer 10 ul from plate 1, well A1 to plate 2, wells A1-A6.
    Transfer 10 ul from plate 1, well A2 to plate 2, wells A7-A12.
    Transfer 10 ul from plate 1, well A3 to plate 2, wells B1-B6.
    Transfer 10 ul from plate 1, well A4 to plate 2, wells B7-B12.

Using a multichannel pipette:
Transfer, in triplicate, 10 ul from patients wells of plate 1 into the corresponding wells of plate two. For example, patient #1 sample from well D1 of plate one is transferred to wells D1-D3 of plate two. Patient #1 sample from well D2 is added to wells D4-D6 of plate two, etc. Continue to add samples in this manner until all Patient samples have been added to plate 2.

11. After transfers are complete, mix all wells of plate two using a multichannel pipette. Be sure to dispense tips between rows.

12. Using a 12-tip multichannel pipette, make direct transfers of 18.5 ul from plate 2 into plate 3 by row. For example, transfer wells A1-A12 of plate 2 into wells A1-A12 of plate 3. Mix plate 3 using a multichannel pipette as explained previously.

13. As in step 12, make a direct transfer of 50 ul from plate 3 into the cell plates prepared before start of assay. Gently add dilution to the cell plate (do not pipette up and down).

14. Incubate the SP2/Tac assay plates 18-20 hours at 37°C. Pulse the cell plates using leucine-free RPMI media and 3H leucine at a dilution of 1:50 (1 uCi/well). Gently add 50 ul of the 1:50 3H leucine dilution to the assay plates using a multichannel pipette. Dispense tips between rows. Incubate the plates for 41/2 hours at 37°C.

15. The cell plates are harvested after incubation using a MACH III Tomtek cell harvester. Harvester instructions located in protocol book in room 428A. The cells are harvested onto filter mats and read using a Wallac Beta Counter and the data is electronically captured.

Reasons to repeat an assay:

1. Failure to get counts above 2500.
2. Failure of the standard curve.
3. Low counts in serum only tube (as compared to subsequent serum dilutions).
4. Dr. Kreitman requests a repeat of assay.

10.5 APPENDIX E: LMB-2 PK ASSAY FOR SP2/TAC CELLS

DAY 1:

1. Plate SP2/Tac cells at 40,000/well in flat bottom plates. A typical PK assay requires 3 plates (I, II, and III).

2. Label 2 U-bottom plates (I & II) and add room temperature SP2/Tac media in the following fashion:
   a. In U-bottom plate I: wells A1-A5 receive 200 ul, wells A6-A7 receive 198 ul/well, A8 receives 205 ul, columns 1-3 starting with row B receive 198 ul, and column 4 receives 205 ul starting with row B.

   b. In U-bottom plate II: wells A1-4 and A7-10 receive 200 ul of media, columns 1-3 and 7-9 starting with row B receive 198 ul, and columns 4 and 10 starting with row B receive 205 ul.

3. Add 8.5 ul of each plasma time point to column 4 starting with row B of U-bottom plate I in sequential order. It is very important to double-check the order of the time points. Day 1 time points are added first in order of time drawn, then day 3, etc. A total of seven time points can be added to U-bottom plate I.

4. Proceed to U-bottom plate II. Additional time points, in sequential order, can be added first to column 4 starting with row B, then to column 10 also starting with row B. Row A is left blank in both U-bottom plates as Row A is used for the titration row. Dr. Kreitman HSA requested a total of two "blank" rows per PK assay. These rows receive only media in the final step of the assay (no toxin or plasma is added to these rows). The assay must be structured so that a total of two rows in the flat bottom plates are not used. A total of 19 time points can be run on three plates. If more than 19 time points are to be run, it will be necessary to use an additional cell plate IV.

5. After the plasma samples are added to the U-bottom plates, an 8 well multichannel pipette is used to the dilutions. The same procedure is used on each U-bottom plate starting with row B. Transfer 22 ul from column 4 into column 3 (i.e. 22 ul from B4 is transferred into B3, etc.). Column 3 is mixed well and 22 ul is transferred from column 3 into column 2. Finally, 22 ul is transferred from column 2 into column 1. The same procedure is used to make dilutions from column 10 through column 7 of U-bottom plate II. It is important that thorough mixing is performed after each dilution and before the next transfer is made.

6. Add 8.5 ul of LMB-2 toxin to well A8 of U-bottom plate I. Mix well. Transfer 22 ul of A8 into A7, 22 ul of A7 into A6, 50 ul of A6 into A5, 50 ul of A5 into A4, 50 ul of A4 into A3, 50 ul of A3 into A2, and 50 ul of A2 into A1. Again, thorough mixing is necessary in each well before each transfer is made.

7. Transfer 50 ul from well A5 of U-bottom plate I into wells A4 and A10 of U-bottom plate II. A series of 50 ul transfers are made from A4 to A3, from A3 to A2, and from A2 to A1 with thorough mixing after transfer. Next, 50 ul transfers are made from A10 to A9, from A9 to A8, and from A8 to A7. This is to assure a titration curve for each of the three cell plates of the PK assay. Once the toxin is added to the U-bottom plates and mixed, the final transfer to the cell plate is made. At this point, there should be three sets of four columns containing serum and toxin dilutions on the U-bottom plates I and II.

8. The PK assay does not require a 15 minute incubation before addition of samples to the cell plates. Therefore, a multichannel pipette is used to transfer a 50 ul aliquot from each column of the U-bottom plates to the SP2/Tac cell plates in triplicate. For example, 50 ul from column 1 of U-bottom plate I is added to columns 1-3 of SP2/Tac cell plate I, column 2 of U-bottom plate I is added to 4-6 of cell plate I, column 3 to columns 7-9, and column 4 to columns 10-12 etc.

9. Similarly, 50 ul from columns 1-4 of U-bottom plate II are transferred to SP2/Tac cell plate II in triplicate and columns 7-10 of U-bottom plate II are added to SP2/Tac cell plate III in triplicate. Remember to add 50 ul of media (without toxin or plasma) to the rows used as blank rows. If media is not added to these rows, the counts will be slightly higher and will not accurately represent the control value.

10. Incubate the plates for 18-20 hours at 37°C. End of DAY 1.

DAY 2:

1. Following overnight incubation at 37°C, the plates are pulsed, harvested, and counted as in the neutralization assay.

Reasons to repeat assay:

1. Failure to get counts above 2500.
2. Failure of standard curve.
3. Draw points run in improper order.
4. Repeat requested by Dr. Kreitman.

INSTITUTE: National Cancer Institute

STUDY NUMBER: 04-C-0142 PRINCIPAL INVESTIGATOR: Robert J. Kreitman, M.D.

STUDY TITLE: A Phase II Clinical Trial of Anti-Tac(Fv)-PE38 (LMB-2) Immunotoxin for Treatment of CD25 Positive Cutaneous T-Cell Lymphomas

Continuing Review Approved by the IRB on 6/27/11
Amendment Approved by the IRB on 8/3/11 (J) Date Posted to Web: 8/16/11
Standard

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 is a phase II clinical trial for the treatment of cutaneous T-cell lymphoma (CTCL) with an experimental drug called LMB-2. The most common types of CTCL are Mycosis Fungoides (MF) and Sézary Syndrome (SS). A phase II trial continues to test the safety of a drug and begins to evaluate how well a new drug works. Phase II trials usually focus on a particular type of cancer. LMB-2 is a biologic therapy drug 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 lymphoma cells must have CD25 on their surface. We plan to include at least 35 patients on this trial.

LMB-2 is an experimental new drug that is considered to be 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 kills the cancer cell to which it binds. The binding part is derived from a protein that is naturally produced by mice. This particular binding protein has been selected because it binds or targets a protein on human cancer cells called CD25. The toxin

portion of LMB-2 is naturally produced by bacteria. LMB-2 is a protein that is produced by recombinant DNA technology. That means that part of the mouse gene responsible for producing the binding protein has been connected to part of the toxin gene and then put into bacteria. The bacteria then make large amounts of LMB-2. We believe that the binding part of LMB-2 will selectively target and kill the cancer cells that have CD25 on their surface. In laboratory experiments, LMB-2 has been shown to kill CD25-containing cells outside a human body and it has caused 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 2 patients with CTCL were treated. In that trial, a partial response was observed in 1 patient with Sézary Syndrome. Another patient with Mycosis Fungoides had stable disease with shrinkage of some of his tumor in the skin and reduction of his itching. Patients with other cancers including Hairy cell leukemia (4 patients), chronic lymphocytic leukemia (1), adult T-cell leukemia/lymphoma (1), and Hodgkin's lymphoma (1) had reduction in their tumors also.

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 (IV) infusion every other day for 3 doses (days 1, 3, 5). You will receive 6 cycles of LMB-2 every 4 weeks unless you develop worsening of disease, serious side effects, or voluntarily withdraw. Up to 3 more cycles may be given to you if you continue to have tumor shrinkage between cycles 4 and 6.

A small amount of blood 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 (if you have lymphoma 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 examination of your skin disease, blood tests, chest Xray, and electrocardiogram (test of your heart). Prior to the first and second cycle you will have a biopsy of the lymphoma on your skin. If this biopsy helps us to better understand how your lymphoma is reacting to LMB-2, we may ask for your permission to biopsy your skin prior to other cycles. Prior to the first cycle you will have a computed tomography (CT) scan and an echocardiogram (ultrasound of your heart). You may also have a bone marrow biopsy (if you have stage II-IV disease) and a nuclear medicine scan. If these studies help us understand how your lymphoma is reacting to 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 the first cycle as an inpatient (admitted to the hospital). Subsequent cycles may be given as outpatients (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.

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. Treatment with single drugs or combination chemotherapy, which may produce a brief response but may have little benefit or long-term control.
   b. Radiation treatment, which sometimes can control tumor growth in local areas such as skin, lymph nodes, 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 disease from local areas such as skin and lymph nodes but is not useful for long-term control when the disease has spread.
   d. Treatment with another agents, such as denileukin diftitox (Ontak™) or bexarotene (Targretin™), which may produce a brief response but may have little benefit or long-term control.
2. Other experimental agents that have not been conclusively demonstrated to be effective.
3. 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.
4. No therapy. If you decide to have no therapy for your disease, therapy may be started at a later time. However, for some patients, waiting to start therapy may decrease the potential to benefit from therapy.

Risks or Discomforts of Participation

In order to determine if you are eligible for this experimental therapy, several tests will have to be done. This period of evaluation 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 biopsies of the tumor tissue in the skin, lymph nodes, or bone marrow.

Administration of LMB-2 will be through a central venous catheter 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, or 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 (as of 2/21/02). On that trial, all side effects of LMB-2 went away when LMB-2 was stopped. In some cases this required additional medical treatments. The following list of side effects includes those seen among 74 adult patients with different types of cancer receiving LMB-2 on different clinical trials:

Possible:

- Decrease in heart's ability to pump blood during the "active" phase of the heartbeat (systole)
- Stiffness in the heart preventing the heart chambers to properly fill with blood
- 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)
- 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
- Decreased levels of a blood protein called albumin
- Muscle weakness
- Abnormal breakdown of muscle fibers, potentially fatal
- 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
- Low blood pressure

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. This can be severe, and although vascular leak syndrome usually gets better, it may require intubation and can be fatal.

Side effects associated with immunotoxins in general 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
- neurologic problems including sleepiness, decreased level of alertness, weakness, painful tingling ("pins and needles"), numbness (decreased feeling), and coma.

A condition known as hemolytic uremic syndrome (HUS) has been seen with related immunotoxin drugs. 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
- a temporary decrease in the platelet count and/or red blood cell count
- Fainting episodes related to needle insertion can occur
- 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)
- 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.

During the early testing of LMB-2, high doses (over 6 times the dose on this study) were given to mice to look for possible side effects. At this high dose half of the mice died of liver damage. In another study, high doses (7.5 times the dose on this study) were given to monkeys to look for side effects. These doses did not cause deaths. Side effects seen included:

- decreased food intake
- weight loss
- laboratory abnormalities indicating liver damage, liver enlargement, and increased white blood cell count
- changes in the liver, skin, testicles, and heart were seen under the microscope.

While animal studies provide some information, different effects may be seen in humans.

It is possible that your body will make antibodies against LMB-2. High levels of such antibodies may neutralize the effect of LMB-2 and prevent it from working. Blood tests will be sent regularly to monitor for antibodies in your case. Studies of a similar drug, denileukin diftitox, have suggested that patients who develop neutralizing antibodies may continue to have benefit from the drug. In addition, those patients did not have an increased risk for side effects or allergic reactions.

Patients with lymphoma 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 over 101ºF (38.3ºC) or any signs of infection.

Risks Associated with Routine Procedures:

Blood Drawing: To monitor the effects of therapy frequent blood tests will be necessary. Up to a pint, or one unit, of blood may be drawn every 6 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.

Biopsy of the tumor tissue in the skin: Local anesthesia will be used if biopsies of tumor masses are performed. Risks involved in skin biopsy are uncommon but include discomfort, bleeding, a thickened scar (i.e., a keloid), and infection. A permanent scar that may or may not be immediately visible will result at the site of the skin excision. Allergy to anesthetic is extremely uncommon but may occur. Your primary care physician or a nurse/physician at the Clinical Center will remove your sutures one to two weeks after your excision.

Central Venous Catheter (CVC): 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.

Premedications:
Acetaminophen (Tylenol): side effects are extremely unlikely. Regular use of acetaminophen can cause liver damage especially at high doses (above 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.

Dexamethasone (Decadron): To be given only before the first of the 3 doses, only for retreatment (cycles 2-6) cycles. Side effects of a single dose of dexamethasone might include difficulty sleeping, increased hunger, and increased blood glucose. Steroids are associated with an increased risk of infection although this is more common when they are used more frequently.

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 B surface antigen positivity are excluded from this trial because the effect of LMB-2 on hepatitis B and/or the immune system is unknown and potentially harmful. 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. Patients with childbearing potential should use adequate birth control measures while on the study.

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 lymphoma 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 lymphoma.

Research Subject's Rights

You will be given a copy of this consent for your records. Participation in this investigational treatment protocol is voluntary, and you can discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled. You are free to ask questions of the staff, and are encouraged to do so. Any significant new findings that relate to your treatment will be discussed with you.

If any publications or presentations result from this trial, your anonymity will be protected to the maximum extent possible. However, qualified representatives of the Food and Drug Administration (FDA), the National Cancer Institute (NCI), and the drug manufacturer may confidentially inspect your patient records during this study.

Payment

You will not be paid for taking part in this study. Your medical care and the costs of the laboratory and radiographic studies done at the Clinical Center, NIH, will be at no expense to you. If your insurance does not cover the expense of study-related blood tests ordered by your local physician, you can be reimbursed for this expense; but your NIH physician must obtain permission for this in advance. The NIH cannot, however, reimburse you for the costs of other types of medical care delivered outside the NIH, even if you are seeking medical attention as a result of side effects from treatment given here, unless your NIH physician secures advance permission for this. Similarly, we do not ordinarily reimburse the costs of diagnostic radiology tests (such as CT scans, MRI, or chest X-rays) done outside the NIH, even if they are done for the purpose of this study. LMB-2 is supplied by the Cancer Therapy and Evaluation Program, NCI.

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.

Photography of skin lesions

Photos of your skin may be taken at your first and subsequent visits to record changes in your skin disease. These may be used for publication, but in that case, you will not be recognizable from the photo. Infrared photographic images may also be obtained for research purposes. This type of photography is not harmful to you and cannot be used to identify you.

Optional Studies (not required):

We would like to keep some of the blood, bone marrow or urine that is collected for future research. These specimen(s), will not be identified by name when sent outside the NIH or stored, only by number. The use of your specimen(s) will be for research purposes only and will not benefit you. It is also possible that the stored [specimen(s), studies] may never be used. Results of research done on your specimen(s) 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 blood, urine, or bone marrow 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 blood, urine, or bone marrow. Then any that remains will be destroyed. 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 blood, urine, and bone marrow may be kept for use in research to learn about, prevent, or treat cancer.

Yes No Initials_____

2. My blood, urine, and bone marrow may be kept for use in research to learn about, prevent or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease).

Yes No Initials_____

3. Someone may contact me in the future to ask permission to use my specimen(s) in new research not included in this consent.

Yes No Initials_____

Samples to be saved for additional tests:

- Neutralizing antibodies: Antibodies a patient might make which block the effect of certain recombinant immunotoxins like LMB-2. Requires about 1 teaspoon of blood.
- Flow cytometry assays to quantify tumor markers on the malignant cells. Requires about 1/2 teaspoon of blood.
- Bone marrow biopsy samples, whether they obtained at NIH or elsewhere, and whether the bone marrow test has already been done or not yet done.
- Cytotoxicity assays. Cutaneous T-cell 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 of blood.
- 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 of blood.
- HLA typing to better understand the immune system in patients with cutaneous T-cell lymphoma. Requires about 1 teaspoon of blood.
- PAX-gene tube: To obtain RNA to study the mechanism of how cutaneous T-cell lymphoma cells form, and to detect very low levels of l cutaneous T-cell lymphoma cells in patients. Requires about 1/2 teaspoon of blood.
- RNA samples can also be used, in an assay called micro-arrays, to study why some patients may not respond as well as others to recombinant immunotoxins like LMB-2. Taken with PaxGene tube.

- Assays which could have an impact on both patients and their children, including studies of genetic cancer risk, will not be done.
- Samples to determine levels of immunotoxin in blood, urine, and other tissues.

Your research blood samples will only be identified by the study code, subject number, visit number and date and time of collection.

Disclosure of potential 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., Building 10 Room 12C420, Telephone: 301-435-1827. You can contact either one through the hospital page operator 301-496-1211. If you have any questions about the use of your tissue for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

You may also call the Clinical Center Patient Representative at 301-496-2626.

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 JUNE 27, 2011 THROUGH JUNE 26, 2012.

Signature of Investigator _____ Date _____ Signature of Witness _____ Date _____

Print Name _____ Print Name _____

INSTITUTE: National Cancer Institute

STUDY NUMBER: 04-C-0142 PRINCIPAL INVESTIGATOR: Robert J. Kreitman, M.D.

STUDY TITLE: A Phase II Clinical Trial of Anti-Tac(Fv)-PE38 (LMB-2) Immunotoxin for Treatment of CD25 Positive Cutaneous T-Cell Lymphomas

Continuing Review Approved by the IRB on 6/27/11
Amendment Approved by the IRB on 8/3/11 (J) Date Posted to Web: 8/16/11
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 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 cutaneous T-cell leukemia cells. To determine your eligibility for LMB-2, we would first need to test your blood, bone marrow, 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. Your blood, bone marrow, tumor

or other tissue may also be tested for other factors for research purposes. However, this consent does not permit any additional studies that would test for genes (i.e. tendency for diseases) that might be inherited from you by your children.

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.

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

- Neutralizing antibodies: Antibodies a patient might make which block the effect of certain recombinant immunotoxins like LMB-2. Requires about 1 teaspoon of blood.
- Flow cytometry assays to quantify tumor markers on the malignant cells. Requires about 1/2 teaspoon of blood.

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

We would like to keep some of the blood, bone marrow or urine that is collected for future research. These specimen(s), will not be identified by name when sent outside the NIH or stored, only by number. The use of your specimen(s) will be for research purposes only and will not benefit you. It is also possible that the stored [specimen(s), studies] may never be used. Results of research done on your specimen(s) 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 blood, urine, or bone marrow 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

blood, urine, or bone marrow. Then any that remains will be destroyed. 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 blood, urine, and bone marrow may be kept for use in research to learn about, prevent, or treat cancer.

Yes No Initials_____

2. My blood, urine, and bone marrow may be kept for use in research to learn about, prevent or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease).

Yes No Initials_____

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You may also call the Clinical Center Patient Representative at 301-496-2626.

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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 JUNE 27, 2011 THROUGH JUNE 26, 2012.

Signature of Investigator _____ Date _____ Signature of Witness _____ Date _____

Print Name _____ Print Name _____