PHASE II STUDY OF THE EFFICACY AND TOXICITY OF ONTAK® (DENILEUKIN DIFTITOX) IN THE THERAPY OF ADULT T-CELL LEUKEMIA

05-C-0185 H

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Precis

Background:

- Adult T-Cell Leukemia is a lymphoproliferative disorder characterized by the presence of CD4/CD25 expressing T cells (IL-2R expressing) in the peripheral blood, in lymphoid and other tissues.
- Denileukin diftitox is a genetically engineered fusion protein combining the enzymatically active domains of diphtheria toxin (DT) and the full length sequence of interleukin-2 (IL-2) that targets IL-2 expressing malignancies.
- Denileukin diftitox interacts with the IL-2 R on the cell surface, is internalized via endocytosis, and inhibits cellular protein synthesis resulting in cell death within hours to days.

Objective:

- Determine the clinical response to Denileukin diftitox **(**Ontak®)of patients with ATL
- Define the safety of Denileukin diftitox in patients who have ATL

Eligibility:

- Patients with chronic, lymphomatous and acute forms of ATL.
- Patients must be HTLV1 positive

Design:

- Nine patients will be treated initially with 9mcg/kg/d of Denileukin diftitox for five days, on an every two week schedule.
- Tumor response will be evaluated after two cycles of treatment. Stable or responding patients will continue treatment with evaluations every four cycles of treatment. Patients will be treated for two cycles beyond a complete remission.
- If no responses are seen at the 9 mcg/kg/d dose, and toxicity is acceptable, an additional 9 patients will be treated at a dose of 18 mcg/kg/d with the same statistical design.
- The trial uses an optimal 2 stage design targeting for a true response proportion > 30%. Nine patients will be treated initially at a dose of 9 mcg/kg/d, with expansion to 29 patients if a response is seen in one of the initial nine patients treated., If no response is seen at the 9 mcg/kg/d dose an additional 9 patietns will be treated at a dose of 18 mcg/kg/d with expansion to 29 patients at this dose level if a response is seen. A stopping rule for excessive toxicity will be incorporated.

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1.1 Study Objectives

1.1.1 Primary Objective

To determine the efficacy of Denileukin diftitox (Ontak®) in the treatment of ATL

1.1.2 Secondary Objectives

To define the safety of Denileukin diftitox (Ontak®) in patients with ATL.

1.2 Background and Rationale:

1.2.1 Epidemiologic Features of ATL

ATL is an aggressive T-cell lymphoproliferative disorder characterized by the presence of malignant CD4/CD25-expressing T cells in the peripheral blood and in lymphoid and other tissues.[1,2] The disease exhibits a striking clustering of cases in certain geographic regions, notably southwestern Japan, the Caribbean basin, northeastern South America, central Africa, and the southeastern United States. Epidemiologic studies demonstrate a clear association of the disease with the presence of a retrovirus, human T-cell lymphotropic virus type I (HTLV-I). The vast majority of HTLV-I-infected individuals are asymptomatic carriers, but infected individuals are nevertheless capable of transmitting the virus.[3] An infected individual appears to have an approximately 0.1 percent per year risk of developing frank ATL; this is equivalent to a cumulative lifetime risk of 2 to 5 percent.

1.2.2 Biology of HTLV-I-Associated ATL

ATL cells exhibit characteristic morphological features (flower-like cells with deeply indented nuclei, derived from mature helper T cells with the surface phenotype $CD3^{+}$dim, $CD4^{+}$, $CD7^{-}$, $CD8^{-}$, $CD25^{+}$). [1,2] ATL cells usually contain chromosomal abnormalities. Although no individual cytogenetic change is characteristic of the disease; the degree of aneuploidy correlates with clinical aggressiveness. The molecular mechanism by which HTLV-I transforms T cells is not fully defined. The HTLV-I genome does not contain an oncogene. Although the site of integration of the provirus is identical in cells taken from a particular individual, it differs from individual to individual; thus the mechanism of transformation is not insertional mutagenesis. The overall genomic structure of HTLV-I is similar to that of other retroviruses. A relatively novel feature of the HTLV-I genome is the presence of a region called pX, which encodes nonvirion proteins including 42-kDa Tax and 27-kDa rex [4]. Deletion of the sequences within these two genes renders an infectious viral clone noninfectious. Tax is a trans-activating transcription factor that activates the HTLV-I long terminal repeat (LTR) and consequently the expression of viral genes under its control. In addition, the Tax protein induces the transcription of various cellular genes including those encoding IL-2Rα, IL-2, IL-3, IL-15, IL-15Rα, TNF, GM-CSF, TGF-1, PTHrP, vimentin, and c-fos.[5] HTLV-I infection of T cells *in vivo* and *in vitro* leads to constitutive IL-2Rα gene expression and facilitates IL-2 expression, thereby leading to T-lymphocyte immortalization. It has been proposed that in the early phases of ATL the HTLV-I-induced leukemogenesis may be the result of Tax expression through its stimulatory effects on genes involved in cellular proliferation. Specifically, Tax expression may begin a process of cellular proliferation via the membrane-localized IL-2/IL-2R interaction with subsequent events

required for frank malignancy. Tax stimulation of other genes such as those encoding GM-CSF, PTHrP and TGF -1 may not be directly involved in the malignant transformation but may result in significant biologic effects.

1.2.3 Clinical Features of ATL

Frank ATL usually has its onset 20-30 years following perinatal infection from the mother infected with HTLV-I. The principal clinical features of full-blown acute ATL include frequent skin involvement, moderate lymphadenopathy with relative sparing of the mediastinum, CNS and lung involvement, and hepatosplenomegaly. [1,2] The peripheral blood may show a high leukemic cell count without much anemia and with only modest involvement of the bone marrow. The occurrence of hypercalcemia, unusual in other types of leukemia and lymphoma, is characteristic of ATL. Patients with ATL manifest a profound degree of immunosuppression and often develop opportunistic infections including Pneumocystis carinii and Cryptococcus meningitis. [6]

The clinical aggressiveness of ATL varies from patient to patient over a very broad spectrum. Investigators have found it useful therefore to subdivide the disease into four categories: 1) Smoldering type whose characteristics are 5 percent or more abnormal T lymphocytes in the peripheral blood in association with a normal lymphocyte level ($<4 \times 10^9$/L), lack of hypercalcemia, lactic dehydrogenase (LDH) values no greater than 1.5 times the normal upper limit, and no lymphadenopathy or organ involvement other than skin and pulmonary lesions. Patients with ATL demonstrable on skin biopsy do not have to manifest 5 percent abnormal cells. 2) Chronic type, absolute lymphocytosis (4×10^9/L or more) with T-cell lymphocytosis more than 3.5×10^9/L, LDH values up to twice the upper limit of normal, and no hypercalcemia or involvement of the central nervous system, bone or gastrointestinal tract or manifestation of associated ascites or pulmonary effusions; 3) Lymphoma type, no lymphocytosis, 1 percent or less abnormal T cells in the circulation, in conjunction with histologically-proven malignant lymphadenopathy; and 4) Acute type, that includes the remaining ATL patients who usually have leukemic manifestations and tumor lesions. [7]

1.2.4 Treatment of ATL

1.2.4.1 Chemotherapy of ATL

Experience with the standard combination chemotherapy regimens known to be useful in the treatment of the more common aggressive non-Hodgkin's lymphomas or acute lymphoblastic leukemia has been very disappointing in ATL. [7] Over the past few years the majority of patients with the more aggressive categories of ATL - acute and lymphoma type - have been treated with combination therapy including CHOP (cyclophosphamide, adriamycin, vincristine, prednisone), VEPA (vincristine, cyclophosphamide, prednisolone, and adriamycin), a Japanese Lymphoma Study Group protocol combining nine drugs (doxorubicin, cyclophosphamide, vincristine, prednisone, vindesine, methotrexate, etoposide, procarbazine, and bleomycin), or a comparable protocol with these drugs plus cisplatin. A total of 854 patients with HTLV-I-antibody-positive ATL newly diagnosed from 1983-1987 were analyzed for prognostic factors and survival following combined chemotherapy by the Japanese Lymphoma Study Group. The median survival time (MST) and projected 2- and 4-year survival rates of all patients were 10 months, 28 percent, and 12 percent, respectively. Impaired performance status, high lactic dehydrogenase

values, age of 40 years or more, increased number of lesions, and hypercalcemia were associated with shortened survival. Survival data has been analyzed according to clinical subtype, with most cases receiving combination chemotherapy. MST was 6.2 months for acute type, 10.2 months for lymphoma type, and 24.3 months for chronic type. Projected 4-year survival rates were 5 percent for acute type, 5.7 percent for lymphoma type, 26.9 percent for chronic type and 58 percent for smoldering type. The members of the Lymphoma Study Group after reviewing 854 cases of ATL under various treatments concluded, "The various combination chemotherapies so far developed have not increased significantly the survival of patients with ATL."

1.2.4.2 Therapy of ATL with AZT/IFN

Because of the generally poor therapeutic results in patients with ATL when conventional combination chemotherapy was employed, new therapeutic strategies are being developed. Currently, several experimental approaches involving the use of radiolabeled MoAbs Deoxycoformycin, interferon and Zidovudine (AZT) are under investigation. In the study by Gill, partial (6) or complete (5) remissions were observed in 11 of 19 patients with ATL treated with a combination of interferon α and AZT. [8] However, the median survival duration of the whole group was only 3 months and was 13 months for the patients who obtained a complete or partial remission.

1.2.4.3 Therapy of ATL with Anti-IL-2Rα-Directed Monoclonal Antibodies

In the Metabolism Branch we have performed a series of therapeutic trials targeting the IL-2Rα expressed on ATL cells. We evaluated the efficacy of the murine monoclonal anti-Tac antibody in the therapy of 19 patients with acute, chronic and lymphomatous ATL (Protocol 83-C-0023). [9] The administration of unmodified murine anti-Tac to these patients produced 2 complete, 4 partial remissions and one mixed response. Subsequently (Protocols 90-C-0043, 93-C-0066) we investigated the efficacy and toxicity of the anti-Tac mAb armed with ^{90}Y. Seven of the 16 evaluable patients so treated achieved a PR and two a CR. [10] Moreover a modified Protocol 96-C-0147 was developed to utilize Hu-anti-Tac rather than Mu-anti-Tac armed with ^{90}Y. Furthermore, study 96-C-0147 permitted the coadministration of Ca-DTPA, a chelate that accelerates the urinary excretion of ^{90}Y. Although 45% of patients responded to treatment during the phase I portion of this trial only one response was seen among nine patients treated with the phase II dose of 25mCi of ^{90}Y Hu-anti-TAC. We are currently evaluating the efficacy of unlabeled Hu-antiTac (Protocol 00-C-0030) administered at high doses in patients with ATL. Doses of 4, 6 and 8 mg/kg have been administered at two and three week intervals. Two of 14 patients treated during the phase I portion of this trial responded to treatment but there were no responses among nine patients in the phase II portion of the trial, although no patients with chronic or smoldering disease were entered in the trial.

1.2.5 ONTAK(Denileukin diftitox)

ONTAK (denileukin diftitox), a recombinant cytotoxic protein composed of the amino acid sequences for diphtheria toxin fragments A and B (Met_1-Thr_{387})-His followed by the sequences for interleukin-2 (IL-2; Ala_1-Thr_{133}), is produced in an *E. coli* expression system. [11] Denileukin diftitox has a molecular weight of 58 kD. Neomycin is used in the fermentation process but is

undetectable in the final product. The product is purified using reverse phase chromatography followed by a multistep diafiltration process.
Denileukin diftitox is a fusion protein designed to direct the cytocidal action of diphtheria toxin to cells which express the IL-2 receptor (IL2R). Ex vivo studies suggest that denileukin diftitox interacts with the IL2R on the cell surface, is internalized via endocytosis, and inhibits cellular protein synthesis resulting in cell death within hours to days.

The human IL2R consists of three different membrane protein subunits: an α-subunit (CD25), a β-subunit (CD 122), and a γ-subunit (CD 132). IL2R may be expressed in cell surface complexes that exhibit low (CD25), intermediate (CD122/CD132) or high (CD25/CD122/CD132) affinity for IL-2. Only the intermediate- and high-affinity complexes permit endocytosis of Denileukin diftitox. [12] The high affinity form of this receptor is usually found only on Treg (suppressor cells) and on activated $CD4^+$ and $CD8^+$ lymphocytes, activated B-lymphocytes and activated macrophages, but not on other normal human tissues. Malignant cells expressing one or more of the subunits of the IL2R are found in certain leukemias and lymphomas, including cutaneous T-cell lymphoma (CTCL).

In vitro studies have been performed which show that CD4+CD25+ cells can be eliminated from human peripheral blood mononuclear cells (PBMC) with the addition of denileukin diftitox. Ex vivo studies suggest that denileukin diftitox interacts with the high affinity IL-2 receptor on the cell surface and inhibits cellular protein synthesis, resulting in cell death within hours. [11] Since CD4+CD25+ lymphocytes are the only cells in the normal resting circulation that express the high affinity IL-2 receptor (all three chains) denileukin diftitox may be effective in eliminating these inhibitory cells. [13-15]

The biodistribution and excretion of radiolabeled denileukin diftitox was evaluated in rats (FDA approved package insert for denileukin diftitox).The liver and kidneys were the primary sites of distribution and accumulation of radiolabeled material outside of the vasculature. Denileukin diftitox was metabolized by proteolytic degradation. Excreted material was less than 25% of the total injected dose and consisted of low molecular weight breakdown products. Pharmacokinetic parameters associated with denileukin diftitox administered as an IV infusion were also determined over a range of doses (3 to 31 mcg/kg/day) in patients with lymphoma. Following the first dose, denileukin diftitox displayed 2-compartment behavior with a distribution phase (half-life approximately 2 to 5 minutes) and a terminal phase (half-life approximately 70 to 80 minutes). Systemic exposure was variable but proportional to dose. Clearance was approximately 1.5 to 2.0 ml/min/kg and the volume of distribution was similar to that of circulating blood (0.06 to 0.08 L/kg). No accumulation was evident between the first and fifth doses. Gender, age and race were introduced into a multivariate analysis with various pharmacokinetic parameters. The limited available data revealed no statistical relationships between these variables. The development of antibodies to denileukin diftitox has been shown to significantly impact clearance rates, but has not been clearly correlated with decreased clinical efficacy. Multiple studies of denileukin diftitox administration have been published and are discussed in the FDA approved package insert for denileukin diftitox and in many publications. [16-22]

1.2.6 Denileukin diftitox Clinical Trial Experience

A randomized, double-blind study was conducted to evaluate doses of 9 or 18 mcg/kg/day in 71 patients with recurrent or persistent, Stage Ib to IVa CTCL. [17] Entry to this study required demonstration of CD25 expression on at least 20% of the cells in any relevant tumor tissue sample (skin biopsy) or circulating cells. Denileukin diftitox was administered as an IV infusion daily for 5 days every 3 weeks. Patients received a median of 6 courses of denileukin diftitox therapy (range 1 to 11). The study population had received a median of 5 prior therapies (range 1 to 12) with 63% of patients entering the trial with Stage IIb or more advanced stage disease. Overall, 30% (95% CI: 18-41%) of patients treated with denileukin diftitox experienced an objective tumor response (50% reduction in tumor burden which was sustained for > 6 weeks). Seven patients (10%) achieved a complete response and 14 patients (20%) achieved a partial response. The overall median duration of response, measured from first day of response, was 4 months with a median duration for complete response of 9 months and for partial response of 4 months.

In another Phase I/II dose-escalation study, 35 patients with Stage Ia to IVb CTCL were treated. Denileukin diftitox was administered as an IV infusion at doses ranging from 3 to 31mcg/kg/day, daily for 5 days every 3 weeks. The overall response rate in patients with CTCL who expressed CD25 was 38% (12 of 32 patients); the complete response rate was 16% and the partial response rate was 22%. There were no responses in 21 patients with Hodgkin's disease. [20]

More than 650 patients have received Denileukin diftitox in clinical studies for a variety of diseases including CTCL, B- and T-cell NHL, Hodgkin's disease, CLL, acute graft versus host disease, rheumatoid arthritis, HIV infection, recent-onset insulin dependent diabetes mellitus or psoriasis. [16-25] Overall, at doses ranging from 0.5 to 31 μg/kg/d, the most common adverse events were fever, nausea/vomiting and other flu-like symptoms; acute hypersensitivity reactions including hypotension, dyspnea, rash, and back pain; a vascular leak syndrome; delayed-onset rash; elevated hepatic transaminases; and hypoalbuminemia. The dose-limiting toxicity for lymphoma patients was defined at the 27 μg/kg/d dose level by persistent, moderate-to-severe nausea, vomiting, fever, chills, and/or asthenia. While the same events were reported at lower dose levels, the severity of the events was greater at doses above 19 μg/kg/d for 5 consecutive days. Although not statistically significant, it appeared that adverse events might be dose-related. In the largest clinical study to date, the Phase III pivotal trial, no patients discontinued the study secondary to lymphopenia, and myelosuppression was uncommon and did not limit treatment. [17] Denileukin diftitox, produced by Eisai Pharmaceuticals, is commercially available and approved by the FDA for administration at 9 or 18mcg/kg/day in patients with lymphomas expressing CD25.

1.2.7 Rationale for using Denileukin diftitox® in the treatment of adult T-cell leukemia/lymphoma

A Phase I-II dose escalation trial of Denileukin diftitox was conducted in patients with various lymphomas expressing the IL-2 receptor. Denileukin diftitox was administered as an infusion daily for five consecutive days, every 21 days. The study included 17 patients with NHL, including seven with low-grade disease, seven with intermediate grade disease, and three with high-grade lymphoma. Ten patients had Stage IV, six patients had Stage III disease, and one patient had Stage II disease. Antitumor effects were observed in 18% (3/17) of patients. Responses were seen in two of the seven patients with low-grade disease and in one of the seven patients with intermediate-grade disease, while none of three patients with high-grade lymphoma

responded. Responses occurred at doses ranging from 6 to 19 μg/kg/day. Response (PR) durations for the two patients with low-grade lymphoma were two and nine months respectively. The patient with intermediate-grade NHL experienced a duration of response (CR) which has lasted greater than 39 months.

In a Phase II open-label, multi-center study, 64 patients with low or intermediate grade B-cell NHL were treated with Denileukin diftitox 18 μg/kg once daily for 5 consecutive days every 3 weeks. Patients had received a median of 5 prior therapies, with 92% having some previous rituximab exposure. Responses were documented in 12% (5/42) of evaluable patients (those who completed at least 2 cycles of therapy and had response assessed). Initial response noted after 2 cycles of therapy in 4 of 5 patients, was not confirmed until after 4 cycles of Denileukin diftitox. [26]

In a recently completed clinical trial, patients with relapsed or refractory, low- and intermediate grade B-cell NHL were treated with Denileukin diftitox. [24] Denileukin diftitox was administered at a dose of 18 μg/kg once daily for 5 consecutive days every 3 weeks for up to 8 cycles. Of 45 patients evaluable for response, all had been previously treated with rituximab, 32 (71%) were refractory to the last chemotherapy treatment, 7 (16%) had failed prior autologous stem cell transplantation, and thrombocytopenia was common with 17 (38%) patients having less than 100,000 platelets/μL and 10 (22%) less than 75,000/μL. Three complete responses (7%) and eight partial responses (18%) were observed, for an overall response rate of 25%. Responses were observed in several NHL subtypes, including patients with diffuse large B-cell lymphoma and follicular lymphoma, 4 of whom had undergone prior autologous stem cell transplantation. The best objective responses were observed after a median of 4 cycles (range 2-8) of treatment. For responding patients, the median time-to-treatment failure was 7 months with a median follow-up of 9.4 months (range 3-18 months), and the projected progression-free survival at 18 months is 30% (95% CI, 0%-64%). Overall, the toxicity profile was similar to that observed in patients with CTCL. Most toxicities were low grade and transient, and little hematologic toxicity was observed.

New approaches to treatment are needed and CD25 presents an attractive target for therapy. Denileukin diftitox binds to CD25, the IL-2 receptor and is internalized. One patient with rapidly proliferating ATL was treated with an excellent response. The WBC decreased by 50% with each of the five daily administrations of Denileukin diftitox, achieving a normal level by the completion of treatment. The response was not maintained however and by three weeks the WBC had recovered to baseline values. This suggests that a more frequent or higher dose regimen may have been effective in producing a sustained response. The treatment was well tolerated with no acute toxicity.

1.2.8 Proposed treatment plan

Patients with the chronic, lymphomatous and acute forms of ATL will be treated with 9 or 18 mcg/kg/d for five days of Denileukin diftitox on an every two week schedule. Nine patients will be treated initially with a dose of 9 mcg/kg/d with expansion to 29 patients if a response is seen in one of the nine initial patients treated. If no response is seen at this dose level, and if no excessive toxicity is seen, an additional 9 patients will be treated at a dose of 18 mcg/kg/d, If a response is seen, this dose level cohort will be expanded to 29 patients. Tumor response will be evaluated after two cycles of treatment. Stable or responding patients will continue treatment

with evaluations after every four cycles of treatment for up to a total of 12 months of therapy. Treatment with Denileukin diftitox, beyond the 12 month time period is at the discretion of the Principal Investigator. Patients will be treated for two cycles beyond a complete remission.

Summary of results of treatment with denileukin diftitox at the 9 mcg/kg/d dose

Nine patients were treated at the 9 mcg/kg/d dose but no objective responses were seen. Evidence of anti-tumor activity was observed in leukemic patients where as evidenced by reductions in leukemic blood counts (Appendix 4). Two potential alternatives were considered; first, prolonged infusions at the same dose for 10 days and second, a higher dose of the agent. Denileukin diftitox was well tolerated in all patients with only one grade 3 non-hematologic toxicity of clinical importance, a suprventricular tachycardia associated with a cardiac traponin leak that resolved with medical management and did not recur with rechallenge. The toxicity observed at the 9 mcg/kg/d dose is summarized in Appendix 5. This proposed amendment will evaluate the other FDA approved dose of denileukin diftitox, 18 mcg/kg/d for 5 days, administered on an every other week basis to rule out a 30% response rate.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

Eligibility Criteria:

2.1 Inclusion Criteria

2.1.1 Patients must have serum antibodies directed to HTLV-I.

2.1.2 All patients must have a histologically confirmed diagnosis of adult T-cell leukemia/lymphoma and more than 10% of the malignant cells must express CD25.

2.1.3 All stages of Tac-expressing adult T cell leukemia except smoldering are eligible: patients with chronic, lymphomatous or acute ATL are eligible. (See appendix 2 for characteristics of patients with the various stages of ATL)

2.1.4 Patients must have measurable disease. All patients with greater than 10% abnormal (i.e. TAC homogenous strongly expressing) PBMC in the peripheral blood will be deemed to have measurable disease.

2.1.5 The patient must have a granulocyte count of at least 1000/mm^3 and a platelet count of greater than or equal to 50,000/mm^3.

2.1.6 Patients must have a creatinine of less than 2.0 mg/dl.

2.1.7 Omission of cytotoxic chemotherapy for ATL for 3 weeks prior to entry into the trial is required. However, patients receiving corticosteroids will be eligible.

2.1.8 Patients must have a life expectancy of greater than 2 months.

2.1.9 Eligible patients must be $\geq$ 18 years old. There is no upper age limit.

2.1.10 Patients must have SGOT and SGPT value $\leq$ 2.5 times the upper limit of normal and bilirubin $\leq$ 3.0/dl. If a liver function test is judged to be elevated due to the underlying ATL, this parameter will be considered an unevaluable parameter for toxicity determinations.
2.1.11 Patients must have a serum albumin $\geq$ 2.5 g/dl
2.1.12 Patients must be able to understand and sign an Informed Consent form.
2.1.13 All patients must use adequate contraception during participation in this trial and for three months after completing therapy.

2.2 Exclusion Criteria

2.2.1 Patients with symptomatic leukemic meningitis will be excluded. However, patients that have both ATL and another HTLV-I-associated disease, tropical spastic paraparesis (TSP), will be included.
2.2.2 Pregnant and nursing patients are not eligible for the study. Because the effects of Denileukin diftitox on the developing fetus are unknown pregnant women will be excluded. Breast-feeding in patients with HTLV-1 infection is contraindicated because of the risk of transmission of the virus to the child. In addition, Denileukin diftitox may be present in breast milk and produce adverse events in the breast-feeding child.
2.2.3 HIV positive patients are excluded from the study. Denileukin diftitox may produce a different pattern of toxicities in immunocompromised individuals.
2.2.4 Patients with Smoldering ATL are excluded.
2.2.5 Patients with serious intercurrent illnesses, past history of a myocardial infarction within 6 months or severe coronary artery disease
2.2.6 Patients who previously received Denileukin diftitox are ineligible.

2.3 Research Eligibility Evaluation

2.3.1 Complete history and physical examination, with documentation of all measurable or evaluable abnormalities in the chart. Photos of skin lesions are required.
2.3.2 Measurable disease of solid lesions such as lymph nodes and skin nodules will be measured and recorded pretherapy as the sum of the products of two greatest perpendicular diameters of all measurable lesions.
2.3.3 Blood: CBC including differential white count and platelet enumeration, reticulocyte count, coagulation profile (PT, PTT), BUN, creatinine, electrolytes, calcium, albumin, phosphorus, sodium, potassium, uric acid, total protein, total bilirubin, direct bilirubin, alkaline phosphatase, SGOT, SGPT, LDH, CPK, amylase, glucose, cholesterol, triglyceride, quantitative immunoglobulins, and complement levels (C3, C4 and total).
2.3.4 Blood will be tested for antibodies to hepatitis B, hepatitis C, HTLV-I, and HIV by standard ELISA assays. CMV serology and antigenemia will be obtained.
2.3.5 Soluble IL-2Rα (Tac antigen) levels in the serum will be measured by ELISA.
2.3.6 Anti-diphtheria toxin antibodies will be performed in the Immunology Department.
2.3.7 Urine: urinalysis will be performed.
2.3.8 Serum Pregnancy test in women of child-bearing potential.
2.3.9 Imaging studies: Chest x-ray and CT scan or ultrasound of the abdomen.
2.3.10 Skin biopsy of clinically suspicious lesions. One to three lesions may be biopsied to confirm the diagnosis of ATL.

2.3.11 Electrocardiogram and Echocardiogram (to estimate ejection fraction).

2.3.12 Bone marrow aspirate and biopsy will be obtained before treatment.

2.3.13 3 cc of EDTA blood (purple top tube) for surface marker studies (FACS analysis) of peripheral blood mononuclear cells is to be sent to Dr. Thomas Fleisher (10/2C410, Ph: 496-4879). Analysis will include quantitation of total T- and B-cells, as well as Tac (IL-2-Rα)-expressing T cells/mm^3. Immunofluorescence staining will be performed using CD3, CD4, CD7, CD8, CD20, anti-Dr, anti-CD25 (either anti-Tac or BD CD25), and 7G7/B6 MoAbs, examining T cell subpopulation. Lymph node aspirates for flow cytometry will be obtained at baseline in patients with easily accessed lymph nodes using the same antibody panel as peripheral blood.

2.3.14 A standard recall skin test panel for example [tetanus 1:5 dilution 0.1 ml, PPD intermediate strength (5 T.U./0.1 ml) 0.1 ml, Candida albicans 0.1 ml] will be placed ID. The results will be read 48-72 hours later and recorded in the chart by a physician or nurse practitioner.

2.3.15 In patients with leukemia the peripheral blood mononuclear cells will be analyzed for HTLV-I integration and for clonal T-cell receptor gene rearrangements by polymerase chain reaction. Blood is to be sent to the Department of Pathology for analysis.

2.3.16 Blood drawing for protocol studies will be limited to 450 cc per 6 weeks.

2.3.17 Lumbar puncture. Patients with neurologic signs or symptoms (e.g., cranial nerve palsies, leg weakness, and bowel or bladder dysfunction) that are not known to be due to some other diagnosis and that could be indicative of CNS involvement by leukemia will receive a lumbar puncture prior to treatment.

2.3.18 All patients will be asked to undergo leukapheresis prior to recieivng their first cycle of therapy and at completion of therapy. The sample shall be sent to John Brady, Lab of Cellular Oncology, for DNA microarray analysis. Patient identifiers will be removed from all specimens, and they will have assigned a unique number and will be labeled with this number. All future processing such as DNA and RNA extraction as well as microarray analysis will refer to this number in order to protect patient privacy. The key linking patient number to identifying patient information will be kept in a secure log with access restricted to the staff of the Brady laboratory, principal investigator and the medically responsible investigator. All specimens will be submitted to John Brady, Building 41, B201, 41 Center Dr , Bethesda, MD 2089-5055. At least 24 hours notice will be provided prior to sending the sample.

2.3.19 Studies must be completed within 4 weeks of study entry, excluding HTLV-1 antibody, which will be counted as positive if obtained from previous studies or HIV and hepatitis viral studies if negative in patients recently enrolled in other NIH protocols within 6 months. Documentation of HTLV1 positivity will be accepted from sources outside the NIH due to the 2 week time frame required for completion of testing at the NIH. Complete blood counts and blood chemistry panels will be repeated within three days before study entry.

2.3.20 Pathologic Evaluation

Diagnostic pathologic specimens will be reviewed by the Hematopathology Section, Laboratory of Pathology, NCI or the Hematology Laboratory, CC. For the subset of patients who do not have circulating abnormal cells a lymph node or skin biopsy will be obtained for diagnostic purposes and to evaluate the expression of the CD25 (Tac) on the malignant cells. Patients with suspected skin involvement from ATL should have skin biopsies. All skin or lymph node biopsies should be submitted fresh in saline to the Hematopathology Section. Biopsy specimens will be processed for routine

histopathology as well as immunohistochemistry in frozen sections and/or flow cytometry for expression of CD25 (Tac) and other lymphocyte surface antigens. Lymph node aspirates will be handled as per the standard procedures of the cytology section. Patients who require a lymph node aspirate or skin biopsy for diagnosis will be entered in the Metabolism Branch screening protocol (97-C-0143) to obtain these samples as they are being performed for eligibility assessment.

2.4 Patient Registration

All patients must be registered on study before beginning therapy. To register a patient for the study, contact Dr. Kevin Conlon (301-402-2913), Dr. Donn Stewart (301-435-8327), Dr. Thomas A. Waldmann (301-496-6653) or one of the other Associate Investigators.
Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://camp.nci.nih.gov/ccr/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 Study Design

Patients with the chronic, lymphomatous and acute forms of ATL will be treated with 9 or 18 mcg/kg/d for five days of Denileukin diftitox, on an every two week schedule. Nine patients will be treated initially with a dose of 9 mcg/kg/d with expansion to 29 patients if a response is seen in one of the nine initial patients treated. If no response is seen at this dose level, and if no excessive toxicity is seen, an additional 9 patients will be treated at a dose of 18 mcg/kg/d. If a response is seen, this dose level cohort will be expanded to 29 patients. Tumor response will be evaluated after the initial two cycles of treatment. If patients are stable or responding, they will continue treatment with evaluations after every four cycles of treatment for a period of 12 months in total. If patients have achieved a complete remission they shall be treated with a further two cycles of consolidation therapy.
The study will be conducted in accordance with the procedures established by CBER and FDA

3.2 Drug Administration

Denileukin diftitox is for intravenous (IV) use only. Treatment of patients may be administered either on an inpatient or outpatient basis. Patients receiving denileukin diftitox at either 9 or 18 mcg/kg/day for 5 days will receive their first cycle as an inpatient, but if toxicities are acceptable, subsequent dosing may be administered as an outpatient. The daily dose is to be infused over 60 minutes.

Patient's baseline weight will be used to determine all doses of Denileukin diftitox®. Dosing will be adjusted if there is a ± 10% change in weight.

Patients will be premedicated with acetaminophen (650 mg po) and diphenhydramine (25-50 mg po), 30-90 minutes prior to each infusion and PRN for symptoms. Meperidine (25-50 mg IV) may be given if chills or rigors develop.

Patients will be monitored with vital signs (temperature, pulse, respiration and blood pressure) before and every 15 minutes during the Denileukin diftitox infusion, and then every 30 minutes for a total of 2 hours after the infusion.

Some patients may develop tumor lysis syndrome. If the patient is considered at risk for tumor lysis syndrome; allopurinol, hydration and alkalinsiation will be administered according to the discretion of the Principal Investigator.

The most common side effects observed are elevated transaminases, hypoalbuminemia, vascular leak syndrome, fatigue and flu-like symptoms. These side effects tend to occur less frequently with further cycles.

If infusional adverse reactions occur, the infusion should be discontinued or the rate should be reduced depending on the severity of the reaction (see Section 4.1). Denileukin diftitox dose may be modified at the discretion of the principal investigator. If the patient is unable to tolerate the 9mcg/kg/day dose it may be reduced to 4.5mcg/kg/day. If the patient is unable to tolerate the 18 mcg/kg/day dose it may be reduced to 9 mcg/kg/d. Allergic reactions to dose administration are possible, therefore appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available and study personnel must be trained to recognize and treat anaphylaxis. If symptom of moderate (grade 2) or severe (grade 3) hypersensitivity persist for longer than 6 hours despite treatment of symptoms, further treatment with Denileukin diftitox should be discontinued. If anaphylaxis occurs at any time, further treatment with Denileukin diftitox must be discontinued.

3.3 Treatment Modifications

Dose limiting toxicities are defined as toxicities attributed to study drug administration requiring discontinuation of drug. These consist of:

- Any episode of life threatening Denileukin Diftitox hypersensitivity.
- Any grade III or IV toxicity not easily reversible to grade 1 by standard supportive measures.

In those cases where a toxicity occurs, which is deemed related to the study drug by the Principal Investigator , additional dosing may occur once the toxicity has resolved by 2 grades or to grade 1. The daily dose may be reduced to 4.5mcg/kg/day, for patients in the 9 mcg/kg/d group, or to 9 mcg/kg/d for patients in the 18 mcg/kg/d group.

Patients with life threatening hypersensitivity reactions or grade III or IV toxicity that does not resolve to grade1, will be taken off treatment but remain on study for follow-up evaluation.

3.4 Protocol Evaluation

3.4.1 During the first cycle of intravenous treatment, patients will have electrolytes, BUN, creatinine, glucose, AST, ALT, Alkaline phosphatase, total bilirubin, LDH, albumin, total protein, calcium and phosphorous performed daily. If toxicities are no greater than grade 2 during the first cycle, metabolic profiles for subsequent cycles shall be performed every other day.

3.4.2 Daily weights and evaluation for peripheral edema and infection will be performed during the 5 days of treatment to monitor for vascular leak syndrome and infection.

3.4.3 EDTA blood (purple top tube) for surface marker studies (FACS analysis) of peripheral blood mononuclear cells is to be sent to Dr. Thomas Fleisher (10/2C410, Ph: 496-4879). Analysis will include determination of total T- and B-cells, as well as Tac (IL-2-Rα)-expressing T cells/mm^3. Immunofluorescence staining will be performed using CD3, CD4, CD7, CD8, and CD20, anti-Dr, anti-CD25 (either anti-Tac or BD CD25), CD45RA, CD62L, CD69, CD71 and 7G7/B6 mAbs at screening, but staining using CD3, CD4, CD7, CD8, and CD20, anti-Dr, anti-CD25 (either anti-Tac or BD CD25), a panel sufficient to assess disease burden will be done with every formal evaluation time point.

3.4.4 Serum samples screening for antibodies to Diphtheria toxin shall be performed at every formal evaluation time point

3.4.5 Serum samples for IL2Rα soluble will be performed at every formal evaluation time point.

3.4.6 Complete evaluation of evaluable lesions with physical examination and appropriate X-rays / scans and biopsies will be performed after the initial 2 cycles of treatment. Stable or responding patients will continue treatment with evaluations every 4 cycles of therapy. Patients will be treated for 2 cycles beyond a complete remission.

The on study evaluations are outlined in Appendix 1 and 2

3.5 Concurrent Therapies:

Patients will receive empiric treatment with trimethoprim/sulfamethoxazole DS (twice daily three times weekly) unless they are allergic to this medication to prevent *Pneumocystis carinii* pneumonia (PCP). If the patient is allergic to trimethoprim or sulfamethoxazole, alternatives include dapsone (100 mg daily), atovoquone (750 mg twice daily) or inhaled pentamidine. Allopurinol therapy shall be administered at the discretion of the PI.

3.6 Surgical Guidelines

Standard skin or lymph node biopsies as well as bone marrow examination are permitted as required to make the diagnosis of ATL and to determine whether the malignant cells express IL-2Rα as required in the Inclusion Criteria.

3.7 Skin Biopsy:

Standard 4-5 mm punch biopsies of any representative skin lesion(s) will be obtained prior to treatment in consultation with the Dermatology Branch. Biopsies will be repeated in consultation with the Dermatology Branch at the time of subsequent treatments as clinically indicated. Any patient achieving a clinical complete response (CR) will under go repeat biopsy of a site(s) of previous known disease as part of the documentation of the CR.

3.8 Bone Marrow Aspirate and Biopsy:

3.8.1 Diagnostic bone marrow aspirate and biopsy will be obtained prior to treatment and as clinically indicated during the course of the patient's treatment. Those patients achieving a complete response (CR) and with previous known bone marrow involvement will under go a repeat bone marrow aspirate and biopsy as part of the documentation of the CR.

3.9 Lymph Node Biopsy/Aspirate:

3.9.1 Patients with superficial lymph nodes deemed suspicious for involvement with tumor or those with the lymphomatous form of the disease (acute adult T-cell lymphoma) will under go a diagnostic biopsy or aspirate prior to treatment. Biopsy specimens may be used for research purposes including but not limited to microarray analysis, establishment of cell lines, and proteomic analysis.

3.10 Off Treatment criteria:

3.10.1 Tumor progression

A patient's treatment in the study will be discontinued after the first 2 cycles of Denileukin diftitox should there be evidence of tumor progression defined as a persistent (at least two determinations) doubling of the peripheral blood leukemic cell count, the development of new lesions, or Calcium elevations that are uncontrolled by conventional therapeutic procedures. Biopsies of lesions in patients with progressive disease are not routinely planned for patients on this study. If a patient shows a discordant response, a biopsy or aspirate of the involved skin or lymph node may be performed.

3.10.2 Any episode of life threatening Denileukin diftitox related hypersensitivity

3.10.3 Any grade III or IV toxicity not easily reversible by standard supportive measures If judged by the PI to be in the best interest of the patient.

All patients who enter the study and discontinue their treatment early, regardless of the reason for stopping protocol treatment, will if at all possible , have a post treatment evaluation one month after the last dose of Denileukin diftitox as outlined in Section 3.12.

3.11 Off Study Criteria

3.11.1 Patient noncompliance or request to withdrawal from the study

3.11.2 Tumor progression and initiation of another treatment.

3.11.3 Completion of the protocol follow up period

3.11.4 Death

3.12 Post-Treatment Evaluation (Follow-up)

3.12.1 After completion or discontinuation of the study treatment, the patients will be serially followed monthly for the first three months and then at three month intervals unless the patient begins treatment for their ATL under the care of another physician.

At these interval visits, the patients will undergo the following evaluations which are described in Section 2:

- history and physical examination
- hematologic, serum studies
- sIL-2Rα
- Urinalysis
- tumor assessment
- flow cytometry

3.12.2 If clinically indicated patients shall undergo the following studies which are described in Section 2:

- skin biopsy
- skin testing.

3.12.3 Patients shall undergo apheresis on completion of therapy and the sample processed by Lab of Celllular Oncology for DNA microarray

3.12.4 After the first year the patients shall be followed every four months for the second year, every six months for the third through fifth year after completion of treatment, and then on a yearly basis.

4 SUPPORTIVE CARE

4.1 Acute Hypersensitivity

Acute hypersensitivity reactions to denileukin diftitox should be managed as outlined below. Resuscitative drugs and equipment should be readily available during administration. The dose may be reduced at the discretion of the Principal investigator.

For mild symptoms: (Localized cutaneous reactions such as mild pruritis, flushing, or rash.) Decrease the rate of infusion until recovery from symptoms, and monitor patient; complete denileukin diftitox infusion at the initial planned rate.
Diphenhydramine 50 mg, may be administered at the discretion of the treating physician.

For moderate symptoms: (Any symptom not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritis, flushing, rash, dyspnea, or hypotension with systolic BP >80 mmHg.) Interrupt denileukin diftitox infusion, administer diphenhydramine 50 mg i.v., and monitor patient until resolution of symptoms. Resume denileukin diftitox infusion after recovery of symptoms. At the discretion of the treating physician, denileukin diftitox infusion may be resumed at one half the initial infusion rate, then increased incrementally to the initial infusion rate. If symptoms develop after resumption of the infusion, at the discretion of the treating physician, additional oral or i.v. antihistamine may be administered. If resolution of symptoms occurs the infusion may be resumed.

For Severe Symptoms (2% of patients): Systolic blood pressure < 80 mmHg, tachycardia, chest pain or tightness, allergic reaction or anaphylaxis. Interrupt denileukin diftitox infusion, administer diphenhydramine 50 mg i.v., and monitor patient until resolution of

symptoms. If anaphylaxis or severe allergic reaction occurs, treat with corticosteroids or epinephrine, as appropriate. The infusion should be discontinued. Treatment with additional denileukin diftitox may be considered at the discretion of the Principal Investigator with steroid pre-medication in those patients whom have shown evidence of disease response.

4.2 Blood Component Support

Platelets and RBC support will be given as medically indicated to maintain an adequate hematocrit and to maintain the platelet count at a level of at least 10,000/mm^3 whenever possible. All administered blood products will be irradiated and depleted of leukocytes.

4.3 Other Medications

With the exception of drugs that would exclude the patient from the protocol (e.g., other investigational anticancer drugs, monoclonal antibodies, chemotherapy, gammaglobulin, drugs that affect lymphocytes), the patients can take other medicines as needed.

5 DATA COLLECTION AND EVALUATION

5.1 Data Collection

Complete quality assurance records must be maintained on each patient treated on the protocol. These records will be kept by the Research Nurse, and should include copies of primary documentation (e.g., laboratory slips, X-ray reports, scan reports, pathology reports, physician notes, etc.) that demonstrate that:

- The patient met each eligibility criterion;
- The signed informed consent was obtained prior to treatment;
- Treatment was given according to the protocol (dated notes about doses given and reasons for any dose modifications);
- Toxicity was assessed according to protocol (laboratory report slips, etc.);
- Response was assessed according to the protocol (X-ray, scan, lab reports, dated notes on measurements, and clinical assessment, as appropriate); and
- Drug Accountability records were kept for each patient.

5.2 Response Criteria

5.2.1 Tests required evaluating response

During the study: follow-up evaluations will be performed at the completion of 2 cycles (4 weeks) of therapy and subsequently after every 4 cycles (8 weeks) thereafter, as outlined in 2.2.1(history and physical examination), 2.2.3 (hematologic, serum studies), 2.2.5 (sIL-2R), 2.2.7 (Urinalysis), 2.2.2 (tumor assessment), and 2.2.13 (flow cytometry) .

After completion of the study treatment: patients will be serially followed monthly or more frequently if clinically indicated. At each monthly visit the patients will undergo the following evaluations, which are defined in Section 2: history and physical examination, hematologic, serum studies, sIL-2Rα, Urinalysis, tumor assessment, flow cytometry and if clinically indicated skin biopsy and skin testing which are defined in Section 2.

Follow up: Following the first three months of follow-up patients will be followed at three month intervals for the first year after completion of treatment, every four months for the second year, every six months for the third through fifth year after completion of treatment, and then on a yearly basis. At each quarterly visit the patients will undergo the following evaluations, which are defined in Section 2: history and physical examination, hematologic, serum studies, sIL-2Rα, Urinalysis, tumor assessment, flow cytometry and if clinically indicated skin biopsy and skin testing which are defined in Section 2.

5.2.2 Parameters to measure response

Response criteria: From the International Workshop to Standardize Response Criteria for Non-Hodgkin's Lymphoma. Responses must last for at least four weeks off treatment.

5.2.2.1 Complete response (CR)

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

5.2.2.2 Complete response unconfirmed (CRUu)

As per complete remission criterion except that if a residual node is greater than 1.5 cm, it must have decreased by greater than 75 percent in the sum of the products of the perpendicular diameters. Lymphocyte aggregates within the bone marrow must be negative for T-cell markers characteristic of adult T-cell leukemia lymphoma.

5.2.2.3 Partial response (PR)

Reduction by $\geq$ 50% of leukemia cell count or $\geq$ 50% reduction in the size of all measurable lesions, and no increase in size of any measurable or evaluable lesion or appearance of new lesion.

5.2.2.4 Stable disease (SD)

5.2.2.5 Less than a partial response with not more than a 25% increase in leukemia cell count, no new lesions, or less than a 25% increase in any measurable.

5.2.2.6 Progressive disease (PD)

Appearance of new lesions, or an increase of 25% or greater in the sum of the product of the perpendicular diameters of the measurable lesions or persistent (at least two determinations) doubling of the peripheral blood leukemic cell count.

5.3 Toxicity Criteria

Toxicity will be assessed according to the NCI Common Toxicity Criteria version 3.0. A copy of the CTC version 3.0 can be downloaded from http://ctep.cancer.gov/reporting/ctc.html.
All appropriate treatment areas should have access to a copy of the CTC version 3.0

5.4 Statistical Section

5.4.1 This study will include patients diagnosed with chronic, lymphomatous or acute ATL. Full lists of inclusion and exclusion criteria are presented in Section 2.2 and 2.3.

5.4.2 We will use an optimal two-stage design {Simon, 1989} to test for early evidence for efficacy based on complete and partial response. The design is based on the following: (a) the response proportion (partial and complete response) will be less than 5% if the treatment is totally ineffective (Po=0.05), (b) the treatment will be considered effective and worthy of future investigation if the true response proportion is > 30% (P1=0.30), (c) a type I error rate of 5% (i.e., the probability of concluding that the treatment is effective if the true response rate is 5% is 0.05), (d) a power of 95% (i.e., the probability of concluding that the treatment is effective if the true response proportion is 30% is 0.95.

In the first stage of the two-stage design, nine patients will be studied. If there are no partial or complete responses observed among the initial nine patients treated with a dose of 9 mcg/kg/d, the study will be expanded to an additional 9 patients at a dose of 18 mcg/kg/d. If one or more of the nine patients respond at either dose level, the study will proceed to a second stage. An additional 20 patients (i.e., a total of 29 patients in the trial) will be studied in the second stage at the same dose level. If four or more out of the 29 patients respond, the treatment will be deemed effective in early phase II development. The treatment will be deemed ineffective if fewer than four respond out of 29 patients.

With this design, there is a 63% chance of proceeding to the 18 mcg/kg/dose , if the response rate at 9 mcg.kg.dose is an ineffective 5%. Similarly, there is a 63% chance of terminating the study if the response rate at 18 mcg/dose/day is 5%.

The major objective of this study is to determine the anti-tumor activity of Denileukin diftitox with regard to response rate, time to progression, and overall survival. Response rate will be based on the number of patients who achieve either a complete or partial response to therapy. Time to progression will be measured from the date of registration until documentation of disease progression. Overall survival will be determined from the date of registration to the event of death or, for surviving patients, censored by the last day patients are known alive.

5.4.3 Antitumor Efficacy:

Tumor status will be evaluated at pre-treatment, after 2 cycles of therapy, and every 8 weeks on study until the development of a complete remission or evidence of disease progression. Post-study assessment of tumor response will be at four weeks after

completion of therapy and every month for the first three months and then at three month intervals for the first year, four month intervals for the second year, six month intervals for the third through fifth years and then yearly thereafter. The objective tumor response (CR+PR) rate and 95% C.I. will be calculated. For responders, duration of response is measured from the response (CR+PR) to the document disease progression or censored at the latest evaluation. Median duration of response will be calculated using Kaplan-Meier method.

Time to disease progression will be measured from the date of registration until documentation of disease progression. Patients who do not experience disease progression will be censored by last day of evaluation or the latest day with known tumor status prior to being lost to follow-up, or the earliest day when patients receive any new cancer therapies, whichever comes first. Patients who have died without disease progression will also be censored by the day of the last evaluation prior to death. Death without a documented progression will not be considered as an event of disease progression. Overall survival will be determined from the date of registration to the event of death or, for surviving patients, censored by the last day patients known alive. Median time to progression and median of overall survival will be estimated using Kaplan-Meier method.

5.4.4 Safety Endpoint

Drug safety will be assessed by examining adverse events, laboratory abnormalities, physical examination and ECOG performance status change from baseline. Adverse events, serious adverse events and abnormal lab values will be graded according NCI CTC criteria. Their occurrence within 30 days from the last study treatment will be summarized for all patients. We will monitor after the 9^{th}, 18^{th}, 27^{th}, and 36^{th} patient. We will stop for toxicity if more than 5 patients experience grade 4 or greater toxicity attributed as possibly, probably or definitely related to Denileukin diftitox after the 9^{th} patient, more than 10 patients experience toxicity after the 18^{th} patient, more than 15 patients experience toxicity after the 27^{th} patient, and more than 20 patients after the 36^{th} patient. For monitoring, toxicity will be defined over the 28 day period after treatment begins. If the probability of toxicity is 1/3, we will have a 5% chance of stopping the trial early based on the toxicity stopping rule. If the probability of toxicity is 0.60, we will have a 73.2% chance of stopping the trial early. If the probability of toxicity is 0.70, we will have a 94.7% chance of stopping early. If at any time during the conduct of the trial two patients die due to toxicity attributed to Denileukin diftitox accrual to the study will be placed on hold and modifications discussed with the sponsor before reopening the study.

5.4.5 We anticipate enrolling 10-12 patients per year into the Denileukin diftitox trial. Thus, if the trial goes to completion (2nd stage) at the 9 mcg/kg/d dose we anticipate enrolling the 29 patients in approximately 2.5 years. If the trial proceeds to completion through the 18 mcg/kg/d dose, a total of 38 patients will be enrolled in approximately 3.5 years.

6 HANDLING OF BLOOD AND TISSUE SAMPLES FOR RESEARCH PURPOSES

6.1 By SAIC Frederick

The Clinical Support Laboratory, SAIC-Frederick, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Upon specimen receipt each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID. An electronic database is used to store information related to patient samples processed by the laboratory. Vial labels do not contain any personal identifier information. Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long term storage. These facilities are operated by Fisher Bioservices, Inc. under subcontract to SAIC-Frederick. Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, typically the protocol Principal Investigator, specifies who has access to the collection.

When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process. The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

7 DATA AND SAFETY MONITORING PLAN

This protocol does not require a DSMB. The Principal Investigator shall monitor the trial by meeting on a monthly basis with the clinical trial team. The regulatory documents and Case Report Forms (CRFs) will be reviewed to verify adherence to good clinical practice procedures and to the protocol. The completeness, consistency and accuracy of the data entered on the CRFs shall be monitored. Copies of protocols, CRFs, original documentation of test results, correspondence, records of informed consent and other documents pertaining to the study will be kept on file in agreement with national regulation. The Principal Investigator is responsible for the safety and welfare of patients under their care and prompt identification, assessment and reporting of adverse events in accordance with protocol requirements. Safety data will be reviewed at other time points in response to adverse events felt to be medically significant.

8 HUMAN SUBJECTS PROTECTIONS

8.1 Rationale for Subject Selection.

HTLV-I-associated ATL is a rare disease in the United States. Therefore, all subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the

eligibility criteria outlined in Section 2.0. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. 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 individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the patient and a signed informed consent document will be obtained.

8.2 Participation of Children

Children will not be permitted to participate in this study although HTLV-I-associated ATL has been noted in individuals as young as nine years of age. There is no information on the toxicity of Denileukin diftitox in children.

8.3 Evaluation of Benefits and Risks/Discomforts

The potential benefits to the subject are that the patient may undergo a partial or complete remission. Denileukin diftitox has demonstrated antitumor activity against CTCL and T cell NHL's. As noted above (1.2) it has been concluded concerning chemotherapy of ATL that "The various combination chemotherapies so far developed have not increased significantly the survival of patients with ATL." Furthermore, although therapy of ATL with AZT/IFN has been associated with partial and complete remissions, the median survival group of the patients treated was only 3 months whereas it was 13 months for patients with a complete or partial remission. Patients treated with Denileukin diftitox may experience infusion-related toxicities which can usually be managed effectively with appropriate pretherapy medication. Denileukin diftitox may cause immune dysfunction that predisposes to opportunistic infections. Prophylactic antibiotic and antiviral agents will be employed to reduce the risk of these complications. Denileukin diftitox is associated with vascular leak syndrome; therefore special precautions shall be undertaken to diagnose and manage this appropriately with supportive care.

8.4 Risks/Benefits Analysis:

As just noted, toxicity due to Denileukin diftitox can be significant. All reasonable therapeutic interventions will be employed to prevent infusion related toxicity and the long-term consequences of immune suppression induced by treatment. There is a prospect for direct benefit for patients in that the use of Denileukin diftitox has led to remissions in other patients with IL-2R expressing malignancies. No cognitively impaired patients will be included in the study.

8.5 Consent and Assent Processes and Documents

NIH Form: NIH-2514-1 Consent to participate in a Clinical Research Study is appended. The informed consent will be discussed with the subject and they will have the opportunity to have all their questions about the treatment and treatment alternatives addressed. Consent for participation will be obtained by the Principal Investigator or an associated investigator. The original consent will be placed in the patient chart and a copy given to the patient. The consent process will be documented in the medical record.

9 SAFETY REPORTING REQUIREMENTS

9.1 Definitions

9.1.1 Adverse Event

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

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

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

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

9.1.2 Suspected adverse reaction

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

9.1.3 Unexpected adverse reaction

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

9.1.4 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.

9.1.5 Disability

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

9.1.6 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.

9.1.7 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.

9.1.8 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.

9.1.9 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 g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

9.2 NCI-IRB Reporting

9.2.1 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.

9.2.2 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.

10 PHARMACEUTICAL INFORMATION

Denileukin Diftitox (Ontak) is manufactured by Eisai Corporation. It will be provided by the Clinical Center Pharmacy Department for the purposes of this study.

For Amendment G, the patient currently on-study will receive the Denileukin Diftitox (Ontak) supplied by Eisai Corporation via the Access Program. The drug will be administered as described in the attached Eisai Protocol Section 9.3.1 Treatments Administered.

10.1 Denileukin Diftitox

10.1.1 Product description

Denileukin Diftitox, a recombinant cytotoxic protein composed of the amino acid sequences for diphtheria toxin fragments A and B (Met_1-Thr_{387})-His followed by the sequences for interleukin-2 (IL-2; Ala_1-Thr_{133}), is produced in an *E. coli* expression system. Denileukin Diftitox has a molecular weight of 58 kD. Neomycin is used in the fermentation process but is undetectable in the final product. The product is purified using reverse phase chromatography followed by a multistep diafiltration process. Denileukin diftitox is a fusion protein designed to direct the cytocidal action of diphtheria toxin to cells which express the IL-2 receptor (IL2R).
Denileukin diftitox is supplied in 150 mcg/ML sterile, frozen solution in a single-use vial NDC 64365-503-01, 6 vials in a package. Each 2 ml vial of denileukin diftitox contains 300 mcg of recombinant denileukin diftitox in a sterile solution of citric acid (20 mM), EDTA (0.05 mM) and polysorbate 20 (<1%) in Water for Injection, USP. The solution has a pH of 6.9 to 7.2.

Other names: DAB_{389}IL-2; Denileukin diftitox

10.1.2 Storage and preparation for injection of Denileukin diftitox

Special Handling:
• Denileukin diftitox must be brought to room temperature, up to 25°C (77°F), before preparing the dose. The vials may be thawed in the refrigerator in 2 to 8°C (36 to 46°F) for not more than 24 hours or at room temperature for 1 to 2 hours. Denileukin diftitox MUST NOT BE HEATED.
• The solution in the vial may be mixed by gentle swirling; DO NOT VIGOROUSLY SHAKE DENILEUKIN DIFTITOX SOLUTION.
• After thawing, a haze may be visible. This haze should clear when the solution is at room temperature.
• Denileukin diftitox solution must not be used unless the solution is clear, colorless and without visible particulate matter.
• DENILEUKIN DIFTITOX MUST NOT BE RE-FROZEN
• USE APPROPRIATE ASEPTIC TECHNIQUE in dilution and administration of Denileukin Diftitox
• Prepare and hold diluted denileukin diftitox in plastic syringes or soft plastic IV bags. DO NOT USE A GLASS CONTAINER because absorption to glass may occur in the dilute state.
• The concentration of denileukin diftitox must be at least15 mcg/mL during all steps in the preparation of the solution for IV infusion. This is best accomplished by withdrawing the calculated dose from the vial(s) and injecting it into an empty IV infusion bag. FOR EACH 1 ML OF DENILEUKIN DIFTITOX FROM THE VIAL(S), NO MORE THAN 9 ML OF STERILE 0.9%SODIUM CHLORIDE WITHOUT PRESERVATIVE SHOULD THEN BE ADDED TO THE IV BAG.

- Do not physically mix denileukin diftitox with other drugs.
- Prepared solutions of denileukin diftitox should be administered within 6 hours, using a syringe pump or IV infusion bag,
- Unused portions of denileukin diftitox should be discarded immediately. Denileukin diftitox is for intravenous (IV) use only.

10.1.3 Stability

Store frozen at or below –10°C. Prepared solutions of denileukin diftitox should be administered within 6 hours, using a syringe pump or IV infusion bag.

10.1.4 Method of administration

The denileukin diftitox dose should be infused over 60 minutes in this study.
DENILEUKIN DIFTITOX SHOULD NOT BE ADMINISTERED AS A BOLUS INJECTION.
Do not physically mix denileukin diftitox with other drugs.
DO NOT ADMISTER DENILEUKIN DIFTITOX THROUGH AN IN-LINE FILTER.
Prepared solutions of denileukin diftitox should be administered within 6 hours.
The FDA approved recommended treatment regimen (one treatment cycle) is 9 or 18 mcg/kg/day administered intravenously for five consecutive days every 21 days. In this study denileukin diftitox at 9/mcg/kg/day or 18 mcg/kg/day shall be infused over 60 minutes for 5 consecutive days every 14 days. If infusional adverse reactions occur, the infusion should be discontinued or the rate should be reduced depending on the severity of the reaction. There is no clinical experience with prolonged infusion times (>80 minutes).

10.1.5 Special handling

No special precautions are warranted. Empty and partial ampoules should be disposed of as biological waste

10.1.6 Expected adverse events

It should be emphasized that the great majority of experience with denileukin diftitox administration has been in patients with refractory leukemias and lymphomas. These patients are at increased risk for toxicities and all of the following descriptions of toxicities are based on denileukin diftitox administration to these patients. Please refer to the package insert for complete reporting of toxicities.

Main Adverse Events:
Acute Hypersensitivity-type Reactions: were reported in 98 of 143 patients (69%) during or within 24 hours of denileukin diftitox infusion; approximately half of the events occurred on the first day of dosing regardless of the treatment cycle. The constellation of symptoms included one or more of the following, defined as the incidence (%) in these 98 patients: hypotension (50%), back pain (30%), dyspnea (28%), vasodilatation (28%), rash (25%), chest pain or tightness (24%), tachycardia (12%), dysphagia or laryngismus (5%), syncope (3%), allergic reaction (1%) or anaphylaxis (1%). These events were severe in 2% of patients.
Vascular Leak Syndrome - This syndrome, characterized by 2 or more of the following 3 symptoms (hypotension, edema, hypoalbuminemia) was reported in 27% (38/143) of patients in the clinical studies. The onset of symptoms in patients with vascular leak syndrome was delayed, usually occurring within the first two weeks of infusion and may

persist or worsen after the cessation of denileukin diftitox. Special caution should be taken in patients with preexisting cardiovascular disease. Weight, edema, blood pressure and serum albumin levels should be carefully monitored on an outpatient basis. This syndrome is usually self-limited and treatment should be used only if clinically indicated.

Infectious Complications: Infections of various types were reported by 48% (69/143) of the study population, of which 23% (16/69) were considered severe although these patients had leukemia and were unusually susceptible to infections. Decreased lymphocyte counts (<900 cells/L) occurred in 34% of lymphoma patients. In general, lymphocyte counts dropped during dosing period (Days 1 to5) and then returned to normal by Day 15. Smaller changes and more rapid recoveries were observed with subsequent courses.

Infusion-associated Reactions: There are two distinct clinical syndromes associated with denileukin diftitox infusion, an acute hypersensitivity-type symptom complex and a flu-like symptom complex. Overall, 69% of patients had infusion-related, hypersensitivity-type symptoms. A flu-like syndrome was experienced by 91% of patients within several hours to days after denileukin diftitox infusion. The symptom complex consists of one or more of the following: fever and/or chills (81%), asthenia (66%), digestive (64%), myalgias (18%) and arthralgias (8%). In the majority of patients, three symptoms were mild to moderate and responded to treatment with antipyretics and/or anti-emetics. Antipyretics and/or anti-emetics were used to relieve flu-like symptoms; however, the usefulness of these agents in ameliorating these toxicities or as prophylactic agents to decrease the incidence of the acute, flu like toxicities has not been prospectively studied.

Gastrointestinal - The onset of diarrhea may be delayed and the duration can be prolonged. Dehydration, usually concurrent with vomiting or anorexia, occurred in 9% of the patients. The majority of transient hepatic transaminase elevations occurred during the first course of denileukin diftitox and were self-limited resolving within two weeks.

Rash - A variety of rashes were reported, including generalized maculopapular, petechial, vesicular bullous, urticarial and/or eczematous with both acute and delayed onset.

Cardiovascular System - Two patients, both of whom had known or suspected pre-existing coronary artery disease, sustained acute myocardial infarctions while on study. Ten additional patients (7%) experienced thrombotic events. Two patients with progressive disease and multiple medical problems experienced deep vein thrombosis. Another patient sustained a deep vein thrombosis and pulmonary embolus during hospitalization for management of congestive heart failure and vascular leak syndrome. One patient with a history of severe peripheral vascular disease sustained an arterial thrombosis. Six patients experienced less severe superficial thrombophlebitis. Thrombotic events were also observed in preclinical animal studies.

Eyes:- Loss of visual acuity usually with loss of color vision with or without retinal pigment mottling has been reported following drug administration. Recovery was reported in some of the affected patients; however most patients reported persistent visual impairment.

Infrequent Serious Adverse Events - The following serious adverse events occurred at an incidence of less than 5%: pancreatitis, acute renal insufficiency, microscopic hematuria, hyperthyroidism and hypothyroidism. Occasional patients experienced tumor lysis syndrome; patients at risk should receive appropriate prophylaxis prior to administration.

Pregnancy: Animal reproduction studies have not been conducted with denileukin diftitox. It is also now known whether denileukin diftitox can cause fetal harm when administered to a pregnant woman of affect reproductive capacity.
Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants, patients receiving denileukin diftitox should discontinue nursing.

Cautions: Women of child-bearing age and fertile man should be advised to use adequate and effective contraception during treatment with Denileukin diftitox and for at least six months after therapy. The effects of Denileukin diftitox on embryogenesis, reproduction, and spermatogenesis are unknown.

10.2 Diphenhydramine

10.2.1 Diphenhydramine will be supplied from commercial sources by the Clinical Center Pharmacy. Diphenhydramine hydrochloride, is an anti-histamine drug having the chemical name 2-Diphenylmethoxy-N,N-dimethylethylamine hydrochloride and has the empirical formula C17H21N0-HCl. It occurs as a white, crystalline powder and is freely soluble in water and alcohol and has a molecular weight of 291.82. Each capsule contains 25mg or 50mg diphenhydramine hydrochloride for oral administration.

10.2.2 Expected adverse events

General toxicities: Urticaria, drug rash, anaphylactic shock, photosensitivity, excessive perspiration, chills, dryness of mouth, nose and throat.

Cardiovascular System: Hypotension, headache, palpitations, tachycardia, extra-systoles.

Hematologic System: Hemolytic anemia, thrombocytopenia, agranulocytosis.

Nervous System: Sedation, sleepiness, dizziness, disturbed co-ordination, fatigue, confusion, restlessness, excitation, nervousness, tremor, irritability, insomnia, euphoria, paresthesia, blurred vision, diplopia, vertigo, tinnitus, acute labyrinthitis, neuritis, convulsions.

GI system: Epigastric distress, anorexia, nausea, vomiting, diarrhea, constipation.

GU system: Urinary frequency, difficult urination, urinary retention, early menses.

Respiratory symptoms: Thickening of bronchial secretions, tightness of chest and wheezing, nasal stuffiness.

The dose of diphenhydramine is 25-50mg, administered orally before each infusion of Denileukin diftitox to help prevent allergic reaction.

10.3 Acetaminophen

Acetaminophen will be supplied from commercial sources within the Clinical Center Pharmacy. Acetaminophen, 4'-hydroxyacetanilide, is a non-opiate, non-salicylate analgesic and anti-pyretic which occurs as a white odorless crystalline powder, possessing a slightly bitter taste. It has the following molecular formula C8H9NO2 with a molecular weight of 151.6

No significant adverse events are expected but patients will be monitored for hepatic toxicity.

The dose of acetaminophen is 650mg, administered orally before each infusion of Denileukin diftitox to help prevent inflammatory responses.

10.4 Trimethoprim-sulfamethoxazole

Trimethoprim-sulfamethoxazole will be supplied from commercial sources by the clinical center pharmacy. More common side effects include: Hives, lack or loss of appetite, nausea, skin rash, vomiting. Less common or rare side effects may include: abdominal pain, allergic reactions, anemia, chills, convulsions, depression, diarrhea, eye irritation, fatigue, fever, hallucinations, headache, hepatitis, inability to fall asleep or stay asleep, inability to urinate, inflammation of the heart muscle, inflammation of the mouth and/or tongue, itching, joint pain, kidney failure, lack of feeling or concern, lack of muscle co-ordination, loss of appetite, low blood sugar, meningitis, muscle pain, nervousness, tinnitus, sensitivity to light, sever skin welts or swelling, skin eruptions, jaundice.

The dose of trimethoprim (160mg)/ sulfamethoxazole (800mg) is one tablet twice daily for three days each week (Monday, Wednesday and Friday).

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12 Appendix 1: Schema for Evaluation for initial 2 cycles

	Screening	CYCLE 1					CYCLE 2 ONWARDS				
		1D1	1D2	1D3	1D4	1D5	D1	D2	D3	D4	D5
H&P,	x						x				
PS	x	x					x				
Biopsy/Asp (1)	x										
Weight	x	x	x	x	x	x	x	x	x	x	x
VS	x	x	x	x	x	x	x	x	x	x	x
CBC/D/PLT	x	x	x	x	x	x	x		x		x
Retic count	x										
PT&PTT	x						x				
Chem &Mg	x	x	x	x	x	x	x	x	x	x	x
Urinanalysis	x	x	x	x	x	x	x	x	x	x	x
HIV & Hepatitis Serology	x										
HTLVI	x										
CMV serology / CMV Ag (2)	x		x					x			
Quantitative Immunoglobulins	x										
Flow cytometry (3)	x										
PCR (4)	x										
Complement	x										
sIL-2R (5)	x										
CXR	x										
CT scans/ Tumor Measurements (6)	x										
BMA&Bx (7)	x										
Std Recall Skin tests (8)	x										
Skin biopsy (9)	x										
Anti-DT antibodies	x										
Apheresis (10)	x										
DRUG ADMINISTRATION											
Denileukin diftitox		**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**X**
Trimethoprim/SUL		x		x		x	x		x		X

FOOTNOTES: ALL TIMEPOINTS CAN BE OBTAINED WITHIN 7 DAYS OF SCHEDULED MEASUREMENT TO ALLOW FOR HOLIDAYS AND OTHER EVENTS

1. Lymph node aspirates for flow cytometry will be obtained at baseline in patients with easily accessible lymph nodes using the same antibody panel as peripheral blood.

2. CMV serology shall be performed at baseline. CMV antigenemia performed at the start of every cycle. Blood must be drawn the morning of the test (Tuesday or Thursday) and in the lab by 10am. In the absence of CMV Ag being processed, CMV PCR will be checked.

3. 3 cc of EDTA blood (purple top tube) for surface marker studies (FACS analysis) of peripheral blood mononuclear cells is to be sent to Dr. Thomas Fleisher (10/2C410, Ph: 496-4879). Analysis will include determination of total T- and B-cells, as well as Tac (IL-2-Rα)-expressing T cells/mm^3. Immunofluorescence staining will be performed using CD3, CD4, CD7, CD8, CD20, anti-Dr, anti-CD25 (either anti-Tac or BD CD25), and 7G7/B6 mAbs at the screening visit. Quantitative assessment of T cell response.

4. The peripheral blood mononuclear cells will be analyzed for HTLV-I integration and for clonal T-cell receptor gene rearrangements by polymerase chain reaction. It shall be performed in the Pathology Department every 4 weeks (2 cycles).

5. sIL-2R (TAC) antigen will be measured in the serum by ELISA prior to initiation of therapy at at each evaluation time point

6. CT scans are to be repeated after the initial two cycles and then every 4 cycles thereafter.

7. Unilateral bone marrow aspirate and biopsy: repeat after treatment only in patients with evidence of bone marrow involvement at presentation, to confirm complete remission.

8. Standard skin recall tests will be performed prior to enrollment on study. They shall be repeated inpatients who have demonstrated evidence of disease response at the discretion of the Principal Investigator.

9. Skin biopsy of clinically suspicious lesions. One to three lesions may be biopsied to confirm the diagnosis of ATL

10. Leukapheresis shall be performed prior to first cycle of therapy, sent to SAIC Frederick for storage.

13 Appendix 2 Overall Schedule for patients

APPENDIX 2 Overall Schedule for patients

CYCLE NUMBER (Each cycle length 14 days)

	Pre	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Informed consent	x																								
H&P	x	X	X	X	X	X	X	X	X	X	X	X	X	X	x	X	X	X	X	X	X	X	X	X	X
PS	x	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	x
LN Biopsy/Asp (1)	x																								
Weight	x	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	x
VS		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC/diff/plt	x	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistries	x	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Retic / PT/ PTT	x	X	X	x	x	X	X	X	X	X	X	X	X	x	x	x	X	X	X	X	X	X	X	X	
Urinanalysis	x	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Antibody levels (2)	x			X x				X				X				X				X				X	
HTLVI and HIV	x																								
HbsAg and HCV	x																								
CMV testing (3)	x	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Quant IgG's	x			X		X		X		X		X		X		X		X		X		X		X	
Flow Cytometry (4)	x			X				X				X				X				X				X	X
Blood complement	x			X				X				X				X				X				X	
PCR (5)	x			X	x	x		X		x		X		x		X		x		X		x		X	
sIL-2Rα (6)	x			X				X				X				X				X				X	
CXR/CT scan (7)	x			X				X				X				X x	X	X	X	X	X	X	X	X x	X
EKG / ECHO (8)	x																								
Tumor measurements (9)	x			X				X				X				X				X				X	

FOOTNOTES: ALL TIMEPOINTS CAN BE OBTAINED WITHIN 7 DAYS OF SCHEDULED MEASUREMENT TO ALLOW FOR HOLIDAYS AND OTHER EVENTS

1. Lymph node aspirates for flow cytometry will be obtained at baseline in patients with easily accessible lymph nodes using the same antibody panel as peripheral blood.

2. Anti DT antibodies shall be checked pre-treatment and at every evaluation time point

3. CMV serology shall be performed at baseline. CMV antigenemia performed at the start of every cycle, blood must be drawn the morning of the test (Tuesday or Thursday) and in the lab by 10am. In the absence of CMV Ag being processed, CMV PCR will be checked.

4. 3 cc of EDTA blood (purple top tube) for surface marker studies (FACS analysis) of peripheral blood mononuclear cells is to be sent to Dr. Thomas Fleisher (10/2C410, Ph: 496-4879). Analysis will include determination of total T- and B-cells, as well as Tac (IL-2-Rα)-expressing T cells/mm^3. Immunofluorescence staining will be performed using CD3, CD4, CD7, CD8, CD20, anti-Dr, anti-CD25 (either anti-Tac or BD CD25) will be performed at formal evaluation points, after 2 cycles and then after every 4 cycles.. Quantitative assessment of T cell response.

5. The peripheral blood mononuclear cells will be analyzed for HTLV-I integration and for clonal T-cell receptor gene rearrangements by polymerase chain reaction. Blood is to be sent to Dr Raphael for analysis every 4 weeks (2 cycles).

6. sIL-2Rα (TAC) antigen will be measured in the serum by ELISA performed pre therapy, after the initial 2 cycles and then after every 4 cycles of treatment.

7. CXR shall be performed at screening. CT scans performed at screening are repeated after the initial two cycles and then every 4 cycles thereafter until evidence of disease progression or 2 cycles past the best response.

8. ECHO shall be performed at baseline to estimate ejection fraction.

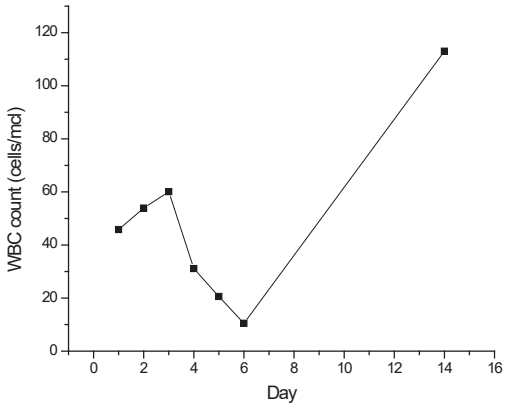
9. Tumor measurements to be performed after the initial 2 cycles then after every 4 cycles of treatment.

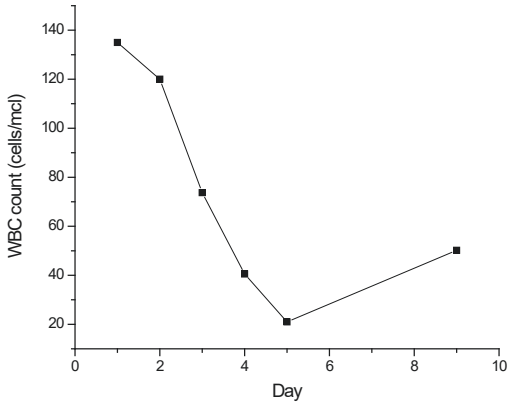
14 Appendix 3

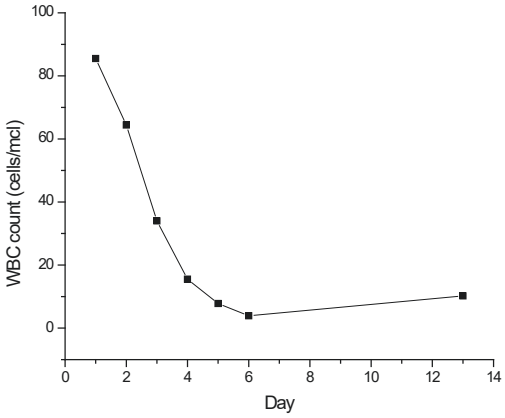
	Smoldering	Chronic	Lymphoma	Acute
HTLV-1 AB	+	+	+	+
Lymphocyte (x10e9)	<4	≥4[a]	<4	*
Abnormal T cells	≥5%	+[b]	≤1%	+[b]
LDH	≤1.5N	≤2N	NA	NA
Ca (mmol/L)	<2.74	<2.74	NA	NA
Histology	No	NA	+	NA
Tumor lesion				
Skin	**	*	*	*
Lung	**	*	*	*
Lymph node	No	*	Yes	*
Liver	No	*	*	*
Spleen	No	*	*	*
CNS	No	No	*	*
Bone	No	No	*	*
Ascites	No	No	*	*
Pleural effusion	No	No	*	*
GI tract	No	No	*	*

*No essential qualification except terms required for other subtypes
**No essential qualifications if other terms are fulfilled, but histology-proven malignant lesion is required in case abnormal T-lymphocytes are less than 5% in peripheral blood
a Accompanied by T-lymphocytosis (3.5 x 10^9/l or more)
b In case abnormal T-lymphocytes are less than 5% in peripheral blood, histology-proven tumor lesion is required.

15 Appendix 4







16 Appendix 5

Grade	1	2	3	4
Hematological				
Neutropenia	1	2	0	1
Anemia	1	4	1	0
Thrombocytopenia	1	2	0	0
Lymphopenia	0	0	4	0
Non-Hematological				
Albumin	0	4	1	0
Alk Phos	2	0	0	0
ALT	1	1	0	0
AST	3	0	1	0
Amylase	1	0	0	0
Bilirubin	2	0	1	0
Creatinine	2	0	0	0
Fatigue	1	0	0	0
Edema	3	0	0	0
Vomiting	3	0	0	0
Cardiac	2	0	0	0
Weight gain	1	0	0	0
Hypotension	1	0	0	0
Hypocalcemia	1	0	0	0
Hypomagnesemia	1	0	0	0
Neuropathy:sensory	1	0	0	0
Proteinuria	1	0	0	0
Mucositis	1	0	0	0
Suprventricular tachycardia	0	0	1	0
Cardiac troponin	0	0	1	0