CLINICAL RESEARCH PROJECT

Protocol #: 06-H-0190
Drug Name: alemtuzumab (Campath®)
IND Number: exempt (5/22/2006)

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To: Richard Cannon, M.D., Chair NHLBI, IRB

Title: Treatment of T-Large Granular Lymphocyte (T-LGL) Lymphoproliferative disorders with alemtuzumab (Campath®)

Other Identifying Words: Neutropenia, anemia, immunosuppression, T-LGL leukemia, chronic T cell lymphocytosis with neutropenia.

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Subjects of Study:	**Number**	**Sex**	**Age range**
	19-39	Either	18-85(both inclusive)

Project involves ionizing radiation?	Yes, as medically necessary
Off-Site project?	No
Multi-Institutional project?	No
DSMB Involvement?	No
Technology Transfer?	Yes

PRECIS

T Cell Large Granular Lymphocyte (T-LGL) lymphoproliferative disorders are a heterogeneous group of uncommon diseases which may involve a polyclonal or a monoclonal T cell population, which bear characteristic surface markers corresponding to activated cytotoxic (CD3+, CD8+) lymphocytes. They are often associated with quite severe neutropenia, anemia, and thrombocytopenia which may be life-threatening. There is some evidence that the abnormal cytotoxic lymphocyte population may cause the cytopenias by suppressing hematopoiesis, although the mechanism is unclear. Immunosuppressive therapy has been shown to improve the cytopenias of T-LGL leukemia, however the long term use of the commonly used agents often lead to significant toxicity in the older patients which are affected by this disease.

Alemtuzumab (Campath®) is currently approved as second line agent in patients with chronic lymphocytic leukemia (CLL) and has been used successfully in the treatment of certain autoimmune disorders. In the Hematology Branch, Campath is currently being investigated in two bone marrow failure syndromes: aplastic anemia and myelodysplasia. Cytopenia(s) is an important characteristic of patients with T-LGL leukemia, often being the indication for immunosuppressive therapy. Our preliminary experience with Campath indicates that it is well tolerated, in particular among the elderly patients.

Therefore, we propose this pilot, Phase II, single agent, study which will evaluate the efficacy and safety of alemtuzumab (Campath®), an immunosuppressive drug, in subjects with T-LGL leukemia. Alemtuzumab (Campath®) will be administered off label at 10 mg per day by intravenous infusion for 10 days total. Subjects who do not show a response to initial Campath or relapse may receive a second cycle of drug after the 3 month time point.

The primary end point of the study is the response rate at three months, defined as improvement in cytopenia(s). Secondary endpoints will include relapse-free survival, response at 6 months, life threatening toxicity, reduction in the number of abnormal T-LGL clone, response to second cycle of Campath, and overall survival.

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1. OBJECTIVES

The primary objective is to identify a possible therapeutic benefit of immunosuppression with alemtuzumab (Campath®) in the treatment of subjects with T Cell Large Granular Lymphocyte (T-LGL) leukemia as evidenced by (a) improvement in cytopenia(s), and (b) a reduction in the number of abnormal T-LGL present in the peripheral blood.

2. BACKGROUND

2.1 Pathophysiology of T-cell large granular lymphocyte (T-LGL) leukemia

A syndrome of increased numbers of circulating large granular lymphocytes (LGL) associated with chronic neutropenia was recognized as a distinct clinical entity in 1977[1]. Since that time, the nomenclature associated with this syndrome has changed many times. In 1993 Loughran proposed a classification based on the demonstration of clonality as well as a characteristic lymphocyte surface phenotype[2]. Clonal LGL proliferations may be either CD3+ (T cell LGL, or T-LGL leukemia) or CD3- (NK cell LGL, or NK-LGL leukemia). T-LGL leukemia is much more common in Europe and in the United States, while NK-LGL leukemia is prevalent in Asia. There is no broad consensus on the exact criteria to establish the diagnosis of T-LGL leukemia, however, there is general agreement that three requirements must be met to establish the diagnosis: (1) evidence of a clonal population of T cells based on T-cell receptor gene rearrangement studies; (2) an abnormal number of phenotypic T-LGLs based on flow cytometry; (3) and the proper clinical scenario of prolonged cytopenia[2,3].

The clinical features of this syndrome, which occurs most commonly in individuals over the age of 50, include recurrent bacterial-infections; occasional splenomegaly, and an association with rheumatoid arthritis.[4,5] Most patients have neutropenia, often severe, and upon inspection of the bone marrow aspirate, a "maturation arrest" in the myeloid series may be seen. Anemia (which may be profound, requiring transfusion support) and thrombocytopenia (usually of moderate degree) occur less frequently. Occasionally, hemolytic anemia has been observed. Lymphadenopathy and skin involvement are distinctly unusual. In the largest single institution cohort of patients, 61 (90%) of 68 patients were alive at 3.5 years, with the majority (70%) requiring therapy during the period of the study. A separate multicenter study reported 20% mortality at 4 years. Spontaneous remissions are rare, if they occur at all.

Examination of the peripheral blood smear is important in establishing the diagnosis of T-LGL leukemia (with the characteristic finding of increased numbers of LGLs) which are characterized by a larger than normal lymphocytes, typically 10 to 20 μm, and abundant pale cytoplasm. Serologic abnormalities are frequent and include polyclonal hypergammaglobulinemia, circulating immune complexes, and positive tests for rheumatoid factor or antinuclear antibody[4]. Less frequently, patients may present with hypogammaglobulinemia, and an occasional patient may have a monoclonal gammopathy[6].

The phenotype of the T cell type of this syndrome is characteristic[2,7]. CD3+ lymphocytes express LGL antigens such as CD16 and CD57 to varying extents. A homogeneous population of CD3+ CD16+ lymphocytes is present in almost all cases of T-LGL leukemia. This subset accounts for fewer than 5% of circulating lymphocytes in normal individuals, and any increase in this population is highly suggestive of T-LGL leukemia. CD56 is uncommonly expressed; however, when present may signal a more aggressive clinical course. Greater than 95% of cases have a TCR αβ+, CD4-, CD8+ phenotype. More recently the

CD52 antigen (the target of Campath) was shown to be highly expressed in malignant cells of patients with T-LGL leukemia, which provides a rationale for the use of Campath in treating this disease[8].

The cause of the LGL proliferation is unknown. CD3+, CD57+ cells, the rare counterpart of the leukemic LGL, are thought to represent in vivo activated cytotoxic lymphocytes (CTL) of unknown antigen specificity[9]. It has been hypothesized that antigen activation plus cytokine secretion could lead to leukemic LGL proliferation in vivo[10]. The mechanism by which neutropenia develops is unclear. T-LGLs may contribute to cytopenia by suppressing normal hematopoiesis directly or by the secretion of cytokines such as interferon-γ and tumor necrosis factor-α. Clonal evolution would depend on additional genetic or environmental factors. Retroviral infection with HTLV-I/II has been proposed as a potential pathway of antigen activation, although serologic positivity against this virus is present in only a few patients[11]. It is also possible that a primary abnormal hematopoietic element may provide the antigenic stimulus for the LGL proliferation, with subsequent myelosuppression resulting from a T cell-mediated attack on the hematopoietic cells. Studies indicate mortality ranging from 10% to 20% at 4 years[7,12].

2.2 Treatment options for T-cell large granular lymphocyte leukemia (T-LGL)

Most patients with T-LGL (>70%) require therapy because of recurrent infections, symptomatic anemia or severe thrombocytopenia. There is no standard approach to the treatment of these patients and optimum treatment remains undefined. The main goal of therapy is to ameliorate the cytopenia(s) to prevent life threatening infections, bleeding or complications from anemia. Case reports and case series in the literature have shown limited responses to steroids, low-dose daily cyclophosphamide, low-dose methotrexate or to granulocyte-colony stimulating factor (G-CSF) [2,13,14,15]. Long-term intermittent treatment with these agents is often required, leading to toxicity and the potential for secondary leukemias or myelodysplasia (e.g., long-term alkylators use). Combination chemotherapy for patients with an aggressive clinical course has not been successful. Three case reports of patients with T-LGL leukemia have documented responses to daily cyclosporine A treatment, which appears to be better tolerated than the aforementioned drugs during potentially long-term therapy.[16,17,18] At NHLBI we treated 25 T-LGL patients with oral cyclosporine and response was observed in about half of the patients. Sustained response required continued low-dose cyclosporine[19]. Despite improvement in the blood counts, increased populations of the CD3+ T-LGLs persisted in patients during cyclosporine therapy, suggesting an amelioration of the cytopenias by the drug as opposed to the eradication of the malignant clone.

2.3 Alemtuzumab (Campath®)

2.3.1 Description

Alemtuzumab (Campath®) is a humanized IgG1 monoclonal antibody directed against the CD52 protein, which is highly expressed on all lymphoid cells and monocytes.[20] Alemtuzumab (Campath®) is a recombinant DNA derived humanized monoclonal antibody that is directed against the 21-28 kD cell surface glycoprotein, CD52. CD52 is expressed on the surface of normal and malignant B and T lymphocytes, NK cells, monocytes, macrophages and tissues of the male reproductive system. Alemtuzumab (Campath®) is an IgG1 kappa with human variable framework and -constant regions and complementarity-determining regions from a murine (rat) monoclonal antibody (Campath-1G). The alemtuzumab (Campath®) antibody has an approximate molecular weight of 150 kD. Alemtuzumab (Campath®) is produced in a mammalian cell (Chinese hamster ovary) suspension culture in a medium containing neomycin. Neomycin is not detectable in the final product. The rationale for using the humanized form of the monoclonal antibody is the decreased formation of antibodies against alemtuzumab (Campath®), which limit efficacy and the potential for re-administration of the drug.

2.3.2 Clinical Pharmacology

Alemtuzumab (Campath®) binds to CD52, a non-modulating antigen that is present on the surface of essentially all B and T lymphocytes, a majority of monocytes, macrophages and NK cells. Analysis of samples collected from multiple volunteers has not identified CD52 expression on erythrocytes or hematopoetic stem cells. The proposed mechanism of action is antibody-dependent lysis of cells following cell surface binding. Alemtuzumab (Campath®) Fab binding was observed in lymphoid tissues and the mononuclear phagocyte system. Significant binding was also observed in the skin and male reproductive tract (epididymis, sperm, seminal vesicle). Mature spermatozoa stain for CD52, but neither spermatogenic cells nor immature spermatozoa show evidence of staining.

Alemtuzumab (Campath®) pharmacokinetics displayed nonlinear elimination kinetics. In CLL patients treated with Campath®, mean half-life was 11 hours (range 2 to 32 hours) after the first 30 mg dose and 6 days (range 1 to 14 days) after the last 30 mg dose. Systemic clearance decreased with repeated administration due to decreased receptor-mediated clearance (i.e. loss of CD52 receptors in the periphery).

2.3.3 Clinical Experience Alemtuzumab (Campath®)

Alemtuzumab produces profound and persistent lymphopenia, affecting predominantly the CD4+ T cell subset. This property has made it attractive in the treatment of a wide range of diseases including rheumatoid arthritis, multiple sclerosis, ocular inflammatory disease, lymphoid malignancies, and in conditioning regimens in stem cell transplantation to prevent graft failure and graft-versus-host disease.[21,22,23,24,25,26,27,28,29,30,31,32]

2.3.3.1 Rheumatoid Arthritis

The mechanisms by which Campath-1H induces its lymphocytotoxic effect appear to be complement-mediated cytotoxicity, antibody-dependent cellular cytotoxicity and induction of apoptosis. Most data regarding the degree and duration of lymphopenia have been derived from patients with rheumatoid arthritis. [33,34] A decrease in lymphocyte and monocyte counts are seen immediately after administration. Initial cell recovery is noticed with CD16+ NK cells and CD14+ monocytes returning to normal levels within 1-2 months, and CD 19+ B cells within 3-6 months. Cytotoxic CD8+ T cell begin to recover at about 3 months after treatment and helper CD4+ T cells may remain suppressed for years. Metteson et al demonstrated that 22% of patients remained lymphopenic at 6 months after treatment, with CD4+ and CD8+ T cells being 14% and 13% pretreatment levels, respectively. [35] Others have shown that T cell subsets can remain suppressed for an even longer period, not rising above 20% of pretreatment levels at any time after 18 months of therapy. [36]

Despite the prolonged and profound lymphopenia, infectious complications in practice have been minor, most occurring in the first 8 weeks and the majority being well controlled and self-limiting. Fatal opportunistic infections have been infrequent.

Campath-1H has been administered in rheumatoid arthritis by subcutaneous and intravenous routes. With the subcutaneous injection, clinical improvement was short- lived, with a high incidence of anti-Campath-1H antibodies. [37] In an intravenous dose-ranging study, 41 patients with RA who had failed at least one disease-modifying anti-rheumatic drug received a total of 100, 250 or 400 mg over 5 or 10 days. [38] Profound lymphopenia developed in all patients with clinical responses appearing more sustained in patients receiving 250 or 400 mg. Approximately 50% of patients

achieved a 50% Paulus response at 31 days and 20% maintained a 50% Paulus response at least 6 months. There were 2 fatal infections, one patient died from infection with *Coccidioides immitis* (methotrexate was added after Campath-1H) and another of suspected hemolytic-uremic syndrome. Overall, infectious complications were minor (mainly herpetic) and all occurred within 8 weeks of therapy.

In rheumatoid arthritis and autoimmune diseases the half-life of Campath-1H has been estimated to be 5 - 9 days. It is likely that the half-life in aplastic anemia will be similar, even though no studies to date have evaluated the pharmacokinetics of Campath-1H in these patients.

Toxicities seen with Campath-1H are short-term infusion-related and long-term due to profound, sustained lymphopenia. Early symptoms include fever, rash, rigors, diarrhea, hypotension and nausea, probably related to cytokine release of TNF-α, interferon-γ, and IL-6. [39] Treatment is mainly symptomatic with discontinuation of the infusion, restarting it as tolerated at a slower rate.

Opportunistic infections are seen most occurring within the first 8 weeks of treatment and have generally been mild. As expected, viral infections with herpes simplex and cytomegalovirus occur at a high frequency. Despite the long duration of CD4+ T cell lymphopenia after treatment with Campath-1H, few fatalities have been reported secondary to opportunistic infections, suggesting that the recovery of B cells and NK cells along with low but existing levels of CD4+ and CD8+ cells are adequate to avoid infectious catastrophes. However, serious, sometimes fatal, opportunistic infections do occur following treatment with Campath-1H.

2.3.3.2 Chronic Lymphocytic Leukemia (CLL)

The efficacy and safety of Campath-1H in CLL was evaluated in a prospective, non-comparative phase II trial conducted at 21 centers in the United States and Europe. [40] Ninety-three patients with high-risk CLL refractory to alkylating agents and fludarabine received Campath-1H 30mg, 3 times a week, up to a maximum of 12 weeks. Overall response was 33%, with 2% complete and 31% partial responses. Median time to progression was 4.7 months, 9.5 months for responders and overall median survival was 16 months, 32 months for responders.

The most common adverse event was infusion-related and was generally grade 1 or 2 in severity. They included rigors, fever, nausea, vomiting and rash. Infections occurred in 51 patients (55%) during the study, being mild to moderate in 26, and more severe (grade 3 or 4) in 25 patients. Septicemia occurred in 14 patients (15%), with grade 3 or 4 in 10 patients. Superficial candidiasis occurred in 9 patients and viral reactivation with cytomegalovirus (n=7) or Herpes simplex (n=6) in 13 patients. A total of 18 patients developed opportunistic infections, 11 during treatment and 7 in the follow-up period. Opportunistic infections included *Pneumocystis jiroveci*(n=1); *Aspergillus* pneumonia (n=1); rhinocerebral mucormycosis (n=1); systemic candidiasis (n=1); cryptococcal pneumonia (n=1, fatal); herpes zoster (n=4, follow-up); pulmonary aspergillosis (n=1, follow-up, fatal); *Listeria* meningitis (n=1, follow-up). The most common opportunistic infection was CMV reactivation (n=7). Nine deaths occurred during treatment or within 30 days of the last administration of Campath-1H (3 progressive disease, 3 pneumonia, 1 pulmonary embolism, 1 sepsis, 1 rhinocerebral mucormycosis) and 19 deaths occurred between 30 and 180 days (11 progressive disease, 6 infections, 1 respiratory distress, 1 inanition). Patients who died during treatment were more likely to have advanced disease at study entry (Rai III or IV) and have failed to respond to Campath-1H.

Most patients experienced transient cytopenias during treatment, with neutropenia most commonly

seen during weeks 5 and 6 (30% of patients) and thrombocytopenia during the first 2 weeks of therapy. Neutrophil and platelet counts recovered by 2 months follow-up.

Two smaller phase II trials in patients with chemotherapy and fludarabine refractory CLL (24 and 29 patients each) also showed similar response rates of about 30%. [41,42] Antimicrobial prophylaxis was not routinely recommended in these trials with infection rates somewhat higher compared to the larger pivotal study. Based on the data from these studies, Campath-1H received FDA approval in May 2001 for patients with B-cell chronic lymphocytic leukemia who have been treated with alkylating agents and have failed fludarabine therapy.

2.3.3.3 Transplant setting to prevent graft rejection and GVHD

Alemtuzumab has also been used as part of conditioning regimens in stem cell transplantation to prevent graft rejection and graft-versus-host disease. [43,44] In solid organ transplantation Campath-1H has been used with cyclosporine in renal allograft recipients to help prevent rejection and reduce further immunosuppressive therapy. [45] In bone marrow transplantation for aplastic anemia, Campath-1G (rat anti-human IgG2b monoclonal antibody directed against the same CD52 antigen) has been used in HLA-identical sibling donors as well as matched unrelated donors with low incidence of graft-versus host disease and graft failures. [46,47]

2.3.3.4 Immune cytopenias

There are no published studies of Campath-1H in patients with aplastic anemia, but a recent case series used Campath-1H in a diverse population of 21 patients with severe autoimmune cytopenias resistant to standard therapy. [48] The diseases included were: 4 with autoimmune neutropenia (AIN), 4 with autoimmune hemolytic anemia (AIHA), -1 with immune thrombocytopenia purpura (ITP), 3 with Evan's syndrome, 3 with autoimmune cytopenias, 1 with ITP and AIN,1 with ITP and pure red cell aplasia (PRCA) and 4 with PRCA. Campath-1H was given as a daily dose of 10 mg intravenously for 10 days. CsA was given to 7 patients and was introduced following Campath-1H. Apart from first dose reactions, Campath-1H was well tolerated in all patients.

Of the 4 patients with AIN, a sustained response was seen in three, with the fourth patient requiring fewer G-CSF injections. Of the 4 patients with AIHA, 2 with warm type autoimmune hemolytic anemia responded and one with cold type hemolytic anemia had a partial response. In the 4 patients with PRCA, 2 responded with one having a relapse after the CsA level was found to be sub-therapeutic. Of the 3 patients with Evan's syndrome, 2 had response but later one relapsed, and the third had a transient response only. A response was seen in 2/3 patients with autoimmune cytopenia, but both relapsed. The one patient with ITP and AIN had a sustained response from the ITP, although he remained neutropenic.

Of the 7 patients who died, 2 had autoimmune cytopenia (1-Guillain Barre syndrome, 1-TTP), 2 had Evan's syndrome (1 – cerebral hemorrhage, 1 – recurrent bronchial carcinoma), 1 had ITP (cerebral hemorrhage), 1 with AIHA (intractable intravascular hemolysis and systemic venous thrombosis), 1 had PRCA (non-Hodgkin's lymphoma transformation). A profound and predictable lymphopenia developed in all patients. B lymphocyte recovery occurred before 3-6 months, CD8+ T cells recovered at variable times ranging from 1-36 months and the median CD4 T cell at last follow up visit was 140 (range 15-560). Despite the lymphopenia there was only one patient who developed a mild viral infection with scattered vesicular skin lesions treated with acyclovir. There was no correlation between CD4 counts and response or relapse.

In a case report, a 51 year old female with a 5 year history of autoimmune neutropenia failed treatment with G-CSF, azathioprine, cyclosporine, one course of horse ATG, one course of rabbit ATG and prednisolone. [49] She was then treated with Campath-1H 10 mg/d for 10 days. Two weeks later, the neutrophil count began to rise and normalized after 2.5 months, which was maintained eight months after treatment.

2.4 Scientific and Clinical Justification

Mature post-thymic T-cell malignancies are a heterogeneous group of rare lymphoid disorders which include T-prolymphocytic leukemia, cutaneous T-cell lymphoma, T-LGL leukemia, and human T-cell lymphotropic virus associated with adult T-cell leukemia-lymphoma. These diseases are often resistant to conventional chemotherapeutic regimens and relapses are common, resulting in patients becoming refractory to treatment. Campath has shown to be active in T-cell malignancies [50]. Anecdoctal reports of successful treatment with Campath in patients with T-LGL leukemia refractory to immunosuppression are emerging in the literature[51,52,53]. An illustrative case was that of a 53-year old woman with T-LGL who was treated with a succession of multiple immunosuppressive and/or chemotherapeutic regimens over 20 months which included methotrexate, prednisone, cyclosporine, cyclophosphamide, anti-thymocyte globulin, OKT3 and pentostatin with no significant sustained response52. Following treatment with Campath, she experienced sustained improvement of the blood counts with minimal toxicity.

Although T-LGL leukemia is commonly thought of as an indolent disease, a majority of patients end up requiring treatment due to constitutional symptoms or complications of cytopenias such as fatigue, bleeding and/or infections[54]. The T-LGL CD3+ clone is frequently resistant to chemotherapeutic agents, and immunosuppression remains the mainstay of therapy. Although small case series indicate that responses are achieved with immunosuppressive agents such as corticosteroids, methotrexate, cyclophosphamide and cyclosporine; relapses are common, and discontinuation of drug and/or long-term intermittent use is often required. This practice often leads to significant toxicity in older patients, which is the age group where T-LGL is most commonly observed. The long term use of alkylating agents such as cyclophosphamide also raises the potential for clonal disorders such as myelodysplasia and leukemia. In addition, eradication of the T-LGL CD3+ clone is infrequent. Campath is a potent lymphocytotoxic agent which is currently approved for the treatment of CLL. The use of Campath in T-LGL may not only improve the cytopenia(s), but also eradicate the leukemic clone and result in more prolonged remission and/or decrease in the rate of relapse.

Anecdotal reports suggest that Campath is active in T-LGL leukemia, with a potential lower toxicity than long term use of corticosteroids, methotrexate or cyclophosphamide. More recently the CD52 antigen (the target of Campath) was shown to be highly expressed in malignant cells of patients with T-LGL leukemia, which provides a rationale for the use of Campath in treating this disease[55].We therefore propose to evaluate the safety and efficacy of Campath in subjects with T-LGL leukemia.

Although T-LGL is classified as a leukemia, tumor burden is not a characteristic of the clinical syndrome; total absolute lymphocyte count is often within normal range or low, lymphadenopathy and/or organomegaly are very infrequently observed. Therefore, the dose of Campath will be the same as it is being used in other bone marrow failure protocols at the Hematology Branch; 10 mg IV for 10 days. When Campath is used as an immunosuppressive agent, doses from 50-100 mg are often used in other scenarios like in bone marrow transplantation and other autoimmune disease (for example, multiple sclerosis). When Campath is used an anti-tumor agent (in chronic lymphocytic leukemia) the dose used is higher; 30 mg three times a week for 8 to 12 weeks (per product label). Since the mechanism of cytopenia(s) in T-LGL is perceived to be similar to other bone marrow failure syndromes, the same

dosing regimen will be used. We have treated 2 patients with T-LGL in the past year who had a complete response following the proposed regimen of 10 mg IV for 10 days.

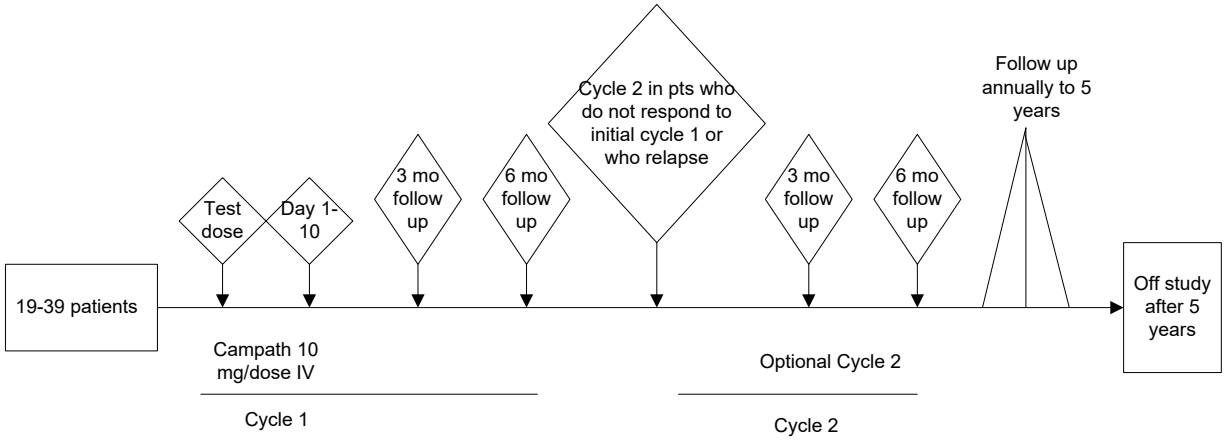
2.5 Justification for a second cycle in subjects with no response or relapsed disease:

We have observed in our preliminary experience that Campath is an active agent in T-LGL and that the regimen is well tolerated with a toxicity profile consistent with the product label (to date no serious treatment related SAEs have been noted in this study). Campath is currently being investigated in several clinical trials worldwide in transplantation and for the treatment of autoimmune diseases including multiple sclerosis where protocol plans include two and sometimes three cycles of Campath [56].

Although we are very encouraged by the initial experience with Campath in T-LGL, response is not universal and some patients are showing signs of relapse after successful initial response. In order to investigate if a repeat course will benefit those who do not respond initially or for patients who respond and relapse, repeating a course of Campath is rational, and it consistent with what is commonly done in other bone marrow failure syndromes like aplastic anemia, where repeat courses of immunosuppression (ATG) are often administered to patients who are refractory or relapse.

3. STUDY DESIGN AND METHODS

This is a pilot study of Phase II clinical trial design to evaluate the safety and efficacy of Campath in subjects with T-LGL leukemia. Alemtuzumab (Campath®) will be administered off label at 10 mg per day by intravenous infusion for 10 days total and then subjects will be monitored for up to 5 years to assess efficacy and safety endpoints. Subjects who do not respond to initial cycle or relapse may receive a second cycle of drug after the 3 month time point.



4. ELIGIBILITY ASSESSMENT

4.1 Inclusion Criteria

4.1.1 Clinical history supportive of the diagnosis of T-LGL leukemia (i.e. a history of cytopenias with

peripheral blood morphologic evidence of LGLs)

4.1.2 Immunophenotypic studies of peripheral blood showing an increased population of T-LGLs (suggested by staining with CD3+, CD8+ and CD16+ or CD57+) or gamma-delta T cells.

4.1.3 Restricted or clonal rearrangement of the T-cell receptor by PCR **AND one or more of the following**

Severe neutropenia (< 500 neutrophils/ μL);

OR

Severe thrombocytopenia (< 20,000 platelets/ μL), or moderate thrombocytopenia (< 50,000 platelets/ μL) with active bleeding;

OR

Symptomatic anemia with a hemoglobin < 9 g/dL or red blood cell transfusion requirement of > 2 units/month for two months prior to initiation of Campath

4.1.4 Age 18-85 (both inclusive)

4.2 Exclusion Criteria

4.2.1 A reactive LGL lymphocytosis to a viral infection

4.2.2 Serologic evidence of HIV infection

4.2.3 Infection not adequately responding to appropriate therapy

4.2.4 Previous immunosuppressive therapy with alemtuzumab

4.2.5 History of carcinoma that is not considered cured (excluding non-melanoma skin carcinoma)

4.2.6 Moribund status or concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the subject's ability to tolerate protocol therapy or that death within 7-10 days is likely.

4.2.7 Current pregnancy, or unwilling to take oral contraceptives or refrain from pregnancy if of childbearing potential

4.2.8 Not able to understand the investigational nature of the study or give informed consent

5 TREATMENT PLAN

5.1. Alemtuzumab (Campath®) Administration

All subjects will initially receive a test dose of 1 mg in 100 ml NS given intravenously over 1 hour.

If tolerated, alemtuzumab (Campath®) will be administered at 10 mg/dose IV for 10 days as an infusion over 2 hours.

Subjects will be admitted to the Clinical Center hospital for study drug initiation. If the study drug infusion is tolerated well (toxicity $\leq$ grade 2) the subject may be discharged and receive the remainder of the treatment course as an outpatient.

5.2 Re-treatment (per PI discretion)

Subjects who do not respond to initial cycle or relapse following initial treatment may be retreated at the discretion of principal investigator with an additional cycle of Campath 10 mg/dose IV for 10 days anytime after the 3 month (primary assessment) time point.

5.3 Pre-medications and management of infusion reactions

Subjects will receive pre-medication with oral diphenhydramine 50 mg and acetaminophen 650 mg 30 minutes prior to infusion.

Infusions reactions will be treated symptomatically (e.g., anti-emetics, IV fluid hydration, acetaminophen, antihistamines, inhaled bronchodilators).

In case of moderate or severe reactions hydrocortisone will be given and the infusion will be discontinued and restarted at a slower rate once the symptoms have subsided.

If a subject has a persistent severe infusion reaction to alemtuzumab (Campath®) that does not respond to measures to ameliorate the signs/symptoms associated to the infusion, the infusion will be discontinued and the subject will be followed for 6 months then taken off study (see section 9.6).

5.4 Permitted supportive care

- Transfusional supportive care (e.g., blood and platelets) as clinically indicated.
- Growth factors if deemed necessary by the investigator or treating physician

5.5 Infection Prophylaxis and Monitoring

- **Pneumocystis prophylaxis:** Aerosolized pentamidine will be used as prophylaxis against *Pneumocystis jiroveci,* 300 mg every 4 weeks by inhalation beginning the first month of therapy and to continue for at least 6 months. If at 6 months CD4+ cells are < 200 /μL, *Pneumocystis jiroveci* prophylaxis will be continued until CD4+ cells > 200 /μL. Trimethoprim/ sulfamethoxazole, Dapsone or other prophylactic regimen against *Pneumocystis carinii* may be substituted at the discretion of the PI.
- **Antiviral prophylaxis:** Valacyclovir 500 mg once daily will be given for at least 2 months to subjects regardless of HSV serology status. If at 2 months CD4+ cells are < 200 /μL, antiviral prophylaxis will be continued until CD4+ cells $\geq$ 200 /μL
- **Antibacterial prophylaxis:** Ciprofloxacin 500 mg BID until ANC > 200 /μL (in subjects with ANC < 200 /μL only). This drug may be continued at the discretion of the PI.

- **EBV and CMV Monitoring:** Subjects will be monitored for EBV and CMV PCR in the blood prior to treatment, then weekly for the first month, every other week in the second month, monthly for another 6 months and at 12 months. In case of a positive PCR for CMV, treatment will be instituted when clinical symptoms are attributed to CMV. EBV positivity by PCR will also be placed in context of clinical signs and symptoms.

5.6 Fever Management of Neutropenic Patients

Subjects with a single temperature of 38.5 °C or two readings of 38.0 °C or greater will be evaluated for infection including cultures of blood and urine and any other suspicious sites prior to beginning empiric therapy. Antibiotics will be initiated following current infectious disease guidelines.

5.7 Instructions to patients

Subjects, who have recently received alemtuzumab (Campath®), should not be immunized with live viral vaccines due to the immunosuppression. In addition, other persons living in the household should not take oral polio vaccine since there is a chance they could pass the polio virus on to the subject. Subjects will be instructed to avoid persons who have taken oral polio vaccine within the last several months, not to get close to them, or stay in the same room with them for very long. If they cannot take these precautions, they will be instructed to consider wearing a protective face mask that covers the nose and mouth.

Subjects who develop hypersensitivity to alemtuzumab (Campath®) will be advised they may have an allergic or hypersensitivity reaction to other monoclonal antibodies.

6 CLINICAL EVALUATION

Bone marrow aspirate will be read by a pathologist and used for diagnostic purposes. Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

Screening evaluations may be performed or obtained from testing performed under 97-H-0041 (Hematology Branch Screening Protocol) or other applicable IRB protocols.

6.1 Pre-study Evaluation

Baseline status will be evaluated as follows:

- Medical History and physical examination
- Baseline laboratory studies
  - Complete blood count with differential
  - Reticulocyte count
  - DAT (direct antiglobulin test)
  - Chem 20 panel
  - Pregnancy test (urine or blood HCG in women of child bearing potential)
  - Folate level
  - B12 level

Coagulation screen (PT, PTT
Iron panel (ferritin, transferrin, % saturation)

- Thyroid function tests
- Viral serologies for hepatitis A, B, C, HIV, HSV, HTLV I/II
- Antinuclear antibody (ANA)
- Rheumatoid factor (RF)
- Serum protein electrophoresis (SPEP)
- Quantitative serum immunoglobulins
- Urine protein electrophoresis (UPEP)
- EBV and CMV PCR in the blood prior to treatment
- Bone marrow aspirate and biopsy with cytogenetic
- Peripheral blood flow cytometry
- T-cell receptor (TCR) gene rearrangement studies
- HLA typing (if not already available)
- Chest X-ray
- Serum troponin before first dose of alemtuzumab (Campath®),
- ECHO, 24 hour Holter monitor before first dose of alemtuzumab (Campath®)
- EKG on the day of admission
- A research apheresis collection (see section 7.0, Lab research studies)

6.2 On study drug monitoring: (cycle 1 and if applicable cycle 2)

On treatment monitoring will consist of:

- CBC with differential (daily)
- Chem 20 panel (every other day)
- Reticulocyte count (weekly +/- 3 days)
- Vital signs (daily)
- ECHO and 24 hour Holter monitor after last dose alemtuzumab (Campath®)
- Serum troponin at day 5 and after the last dose of alemtuzumab (Campath®)
- EBV and CMV PCR in the blood (weekly +/- 3 days)
- Chest x-ray, CT scans or appropriate imaging studies (only as medically indicated)

6.3 Post treatment monitoring discharge home through 3 months: After completing treatment, subjects will be followed by their home physician or at the Clinical Center and have blood work done as detailed below. Progress notes and laboratory results from home physicians will be faxed to Olga Rios, R.N., Research Nurse. Subjects must be evaluated at the Clinical Center at the 3 month ($\pm$7 days) time point

- Interim Assessment
- Complete blood counts with differential (weekly +/- 3 days)
- DAT (NIH) or Coombs test (monthly +/- 2 weeks)
- Chem 20 panel (weekly +/- 3 days) (Home MDs: electrolytes, transaminases, urea nitrogen (BUN), serum creatinine, total bilirubin)
- EBV and CMV PCR in the blood (weekly +/- 3 days) for the first month, then every other week +/- 3 days) in the second month, and then monthly (+/- 2 weeks)
- Reticulocyte count (at 3 months only +/- 2 wks, home MDs only if available)
- ECHO (at 3 months only +/- 2 wks)
- Serum troponin (at 3 months only +/- 2 wks)

- Thyroid function tests (at 3 months only +/- 2 wks)
- Peripheral blood flow cytometry (at 3 months only +/- 2 wks)

6.4 Post treatment monitoring 3 months to 6 months

After the 3 month visit, subjects will be followed by their home physician or at the Clinical Center and have blood work done as detailed below. Progress notes and laboratory results from home physicians will be faxed to Olga Rios, R.N., Research Nurse. Subjects must be evaluated at the Clinical Center at the 6 month (+/-2 wks) time point

- Complete blood counts with differential (every other week +/- 3 days)
- DAT (NIH) or Coombs test
- Chem 20 panel (every other week +/- 3 days) (Home MDs: electrolytes, transaminases, urea nitrogen (BUN), serum creatinine, total bilirubin)
- EBV and CMV PCR in the blood monthly (+/- 2 weeks) to 6 months.
- Reticulocyte count (at 6 months only, Home MDs only if available)
- Thyroid function tests (at 6 months only (+/- 1 month)
- Bone marrow biopsy and aspiration with cytogenetics (6 months (+/- 1 month only)
- Peripheral blood flow cytometry (6 months (+/- 1 month only)
- A repeat research apheresis collection (see section 7.0, Lab research studies)

6.5 Long term Follow up after 6 months

After the 6 month visit, subjects must be evaluated at the Clinical Center (12 months (+/- 2 month) and then yearly (+/- 2 month) thereafter for 5 years and have blood work done as detailed below. Subjects will be seen by their home physician as clinically indicated and the Clinical Center and home physician will continue to communicate.

- Complete blood counts with differential (annually +/- 2 month)
- Chem 20 (annually +/- 2 month)
- Reticulocyte counts (annually +/- 2 month)
- Flow cytometry of the peripheral blood (annually +/- 2 month)
- EBV and CMV PCR in the blood (at 12 months only +/- 2 month)
- Thyroid function tests (annually +/- 2 month)
- Bone marrow biopsy and aspiration with cytogenetics (at 12 months +/- 2 month and annually thereafter, as clinically indicated)

7 ANCILLARY LABORATORY RESEARCH STUDIES

7.1 Collection of samples: During the course of participating on this study, blood and tissue may be collected for correlative laboratory research studies as follows:

- Peripheral blood: 60 cc of blood (at baseline), 20 cc of blood (with EBV/CMV monitoring, at 3mo, 6 mo and annually thereafter (repeated in the event of a 2nd cycle),
- Bone marrow aspirate: 5 -15 cc of bone marrow aspirate (baseline, 6 months, 12 months, and annually thereafter.

- Apheresis: One apheresis collection of approximately 10^{10} leukocytes in a volume of 200 ml prior to initiation of Campath and a repeat apheresis procedure when there is evidence of lymphocyte recovery.

7.2 **Intended use:** These specimens will not be read by a pathologist or used for diagnostic purposes. The studies will not be used in assessing the primary endpoint but are undertaken for descriptive or exploratory ancillary research. These ancillary studies may be done and if done, may be correlated with the presence or absence of response to protocol therapy:

- Characterize LGL cells on a molecular level using gene expression profiling and T-cell receptor genotyping.
- Characterize clonal expansions in LGL by V-beta profile and/or TCR-beta chain sequencing in the peripheral blood and/or bone marrow; identify the epitope by using PS-SCL.
- Flow cytometric analysis of lymphocyte subsets
- Measurement of lymphocyte function and immune response directed to EBV and CMV
- Chromosome analysis using standard cytogenetics and FISH
- Analysis of the T cell receptor V-beta profile in the marrow and peripheral blood of responders and non-responders.
- Clonality analysis using X-chromosome gene inactivation analysis
- HLA typing to see if HLA type correlates with response.
- Evaluation for the presence of abnormalities of the telomere complex
- Assay for cytokines/chemokines and their receptors
- Serum (or plasma) and cells for viral analysis
- Hematopoietic progenitor colony formation and long term-culture-initiating cell assays
- Serum (or plasma) and cells for DNA/RNA extraction and PCR for viral nucleic acids

Campath specific studies that may be done include:

- Anti-alemtuzumab (Campath®) antibody
- Serum levels alemtuzumab (Campath®) may be measured after the last dose and after 1 month from completing the infusion. If done, The alemtuzumab (Campath®) assays will be sent for batch processing to the laboratory of Dr. Geoff Hale, BioAnaLab Limited, Oxford BioBusiness Centre, Littlemore Park, Littlemore, Oxford OX4 4SS. There is a human Material Transfer Agreement (MTA) executed between Cardiff University and NHLBI. This MTA is to send PBMC for the identification of targets (antigens) recognized by LGL clone. No transfer of

material will be accomplished until a Material Transfer Agreement is fully executed through the NHLBI Office of Technology Transfer and Development (OTTAD).

In the event there is sample left over, with the subject's permission, they will be stored for other exploratory laboratory research studies previously reviewed and approved by the NHLBI as listed in Appendix A.

7.3 **Storage:** Research samples will be stored with identifiers in the secure laboratory of the principal investigator.

7.4 **Tracking:** Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside NIH without IRB notification and an executed MTA.

7.5 **End of study procedures**: Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

7.6 **Loss or destruction of samples**: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

7.7 Technology Transfer

This protocol has no associated patents, CRADAs or CTAs. If done, the alemtuzumab (Campath®) assays will be sent for batch processing to the laboratory of Dr. Geoff Hale, BioAnaLab Limited, Oxford BioBusiness Centre, Littlemore Park, Littlemore, Oxford OX4 4SS. There is a human MTA executed between Cardiff University and NHLBI. No transfer of material will be accomplished until a Material Transfer Agreement is fully executed through the NHLBI Office of Technology Transfer and Development (OTTAD).

8 BIOSTATISTICAL CONSIDERATIONS

8.1 Primary Endpoint

The primary endpoint is hematologic response measured at 3 months after first dose alemtuzumab (Campath®) sustained on at least two serial measurements performed 1 week apart and sustained for 1 month or more without support of exogenous growth factors or transfusions.

Complete Hematologic Response: normalization of all affected cell lines

Partial Hematologic Response: Improvement in one or more of the listed parameter (a, b, c) but not sufficient to achieve a complete hematologic response

a) ANC : a 100% increase in ANC to an ANC > 500 /μL

b) Platelets:

For baseline platelet count ≥ 20,000 /μL but ≤ 50,000/μL: a 100% increase in platelet count to a platelet count > 50,000 /μL

For baseline platelet count < 20,000 /μL: a 100% increase in platelet count to a platelet count > 20,000 /μL

c) Hemoglobin: Any increase in hemoglobin by 2 g/dl

8.2 Secondary Endpoints

8.2.1 Transfusion-Independence for red blood cells and/or platelets

8.2.2 Overall survival. In comparing the probabilities of survival between responders and non-responders, the baseline time is set to be 3 months after first dose alemtuzumab at which response is determined for the primary endpoint.

8.2.3 Life Threatening Toxicity. Subjects who do not complete the treatment course because of toxicity or voluntary withdrawal will be included in analyses.

8.2.4 Relapse-free survival is defined as the time elapsed between time of documented response to Campath and evidence for relapse. Relapse is defined as a fall in peripheral blood counts to 50% the values obtained during the response period.

8.2.5 Molecular Response to Campath is defined as disappearance of the clonal population of T-LGL

8.2.6 Response at 6 months

8.2.7 Response to a second cycle of Campath

8.3 Sample size

Because the efficacy of this treatment is almost completely unknown, we would like to reject the treatment as quickly as possible with a small number of subjects if the treatment is not effective. A response probability of 30% or less would warrant terminating the treatment on this patient population, and we hypothesize that the actual response probability using this treatment would reach 50% or more.

Let p be the probability of complete or partial response at 3 months. Our sample size is determined by testing the null hypothesis H_0: p≤30% versus the alternative H_1: p ≥50% at 0.05 significance level and 0.80 of the power. We determine the sample size using the Two-Stage Minimax Design outlined in Table 1 of Simon (1989)[57]. This design is selected because it requires a smaller total number of subjects (n=39) than the Simon's Two-Stage Optimal Design (n=46). At the first stage, 19 subjects will be accrued and the null hypothesis will be accepted (i.e., the treatment will be terminated) if 6 or less of the subjects respond to the treatment at 3 months. If 7 or more subjects respond to the treatment at 3 months at the first stage, then an additional 20 subjects will be accrued, bringing the total number of subjects to n=39. The null hypothesis of p ≤ 30% will be accepted if the total number of complete and partial responders at 3 months is 16 or less.

8.4 Statistical Methods

The planned analyses will include descriptive statistics on the proportions of responses (i.e. % subjects with partial or complete response) and the time to response. The response probabilities will be estimated using the sample proportions and their inferences including confidence intervals and hypotheses testing will be evaluated using Binomial distributions.

The time to responses and the relapse-free survival will be analyzed using appropriate tools in survival analysis, such as cumulative incidence estimate and Cox regression type analysis for covariates, which takes into consideration both death without the event of interest as a complete risk and random censoring due to loss of follow-up. The Kaplan-Meier estimates and Cox regression will be used to evaluate the treatment effects on the overall survival. Graphical tools will be used to display the appropriate estimates (i.e. estimated proportions, the cumulative incidence curves, Kaplan-Meier curves) and their corresponding 95% confidence intervals.

Methods based on multiple regression, analysis of variance, and logistic regression will also be employed if deemed appropriate.

8.5 Stopping rules

The following two types of treatment related severe adverse events will be monitored and considered for early stopping for all or part of the study:

- Death considered to be definitely related to alemtuzumab (Campath-1H®)
- Any grade IV toxicity considered to be definitely related to alemtuzumab (Campath-1H®), i.e. opportunistic infection such as tissue-invasive CMV or Pneumocystis jiroveci, with exception of temporary cytopenias.

We will monitor the numbers of subjects who have developed one or more of the above treatment related severe adverse events (TRSAE). The study will be seriously considered for early stopping if the corresponding number of subjects who have developed one or more of the above TRSAE (Death or any grade IV toxicity considered definitely related to Campath) is over a pre-specified threshold value.

From our experience using this agent in other clinical settings, we anticipate the rate of developing at least one of the above TRSAE to be 10% or less within the first 6 months after first dose of study medication. Following Geller et al. (2003, "Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach"), our stopping rule is determined by a Bayesian approach.[58] The stopping boundary is reached if the Bayesian posterior probability that the true probability of developing one or more of the above TRSAE exceeds this benchmark rate of 10% is at least 90%. We take our prior distribution to be a beta distribution with the sum of the two beta parameters to be 5, i.e. the parameters of the beta prior distribution are 0.50 and 4.50. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring when 3 or more subjects have developed TRSAE. The following table summarizes the threshold numbers for stopping an experiment:

Number of Subjects in the experiment	Stop if the number of subjects who have developed any of the TRSAE reaches:
≤ 10	3
≤ 17	4
≤ 24	5
≤ 32	6
≤39	7

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 39 independent Bernoulli trials, each had a probability p for having the above TRSAE and q=1-p for not having such TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000) which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for p:

Prob of TRSAE = p	2%	5%	10%	15%	20%	25%
Proportion of Stopped Studies	0.1%	2.0%	17.5%	47.6%	75.4%	91.6%
Average Number of Subjects	38.96	38.5	35.2	28.8	21.8	16.0
Average Numbers of TRSAEs suffered by subjects	0.78	1.9	3.5	4.3	4.4	4.0

These results suggest that our stopping rule has a low probability of stopping a study when the proportion of the above TRSAE is below the benchmark value of 10%, and the probability of stopping a study is high when the true proportion of the above TRSAE exceeds this benchmark value. Based on these results, we believe that our Bayesian stopping rule has satisfactory statistical properties.

8.6 Off study criteria

Per Subject choice: Subjects may be removed from study at their request. The risks of withdrawing will be discussed, as will alternative treatment options.

Per principal investigator decision: Alemtuzumab (Campath®) administration will be discontinued; the subject will be followed until the event has resolved if the subject develops:

- Intolerance of alemtuzumab (Campath®) infusion as manifested by hypotension, fever, chills and/or rash that is refractory to supportive measures; OR any subject who develops CTCAE grade 4 hepatic injury or DIC subsequent to infusions of alemtuzumab.
- Life threatening acute hypersensitivity reactions
- Pregnancy or unwillingness to refrain from pregnancy
- Initiation of additional immunosuppressive therapy (except steroids or CsA)
- Failure to respond to Campath therapy
- Subject non-compliance
- Lost to follow-up

- Study completion

Those subjects who choose to withdraw or are removed from the study per PI decision will be asked to continue to have labs monitored through the 6 month off drug time point so that we can continue to monitor for post Campath related safety issues. Once off study subjects will be referred back to his or her referring physician or consented to the Hematology Branch evaluation and treatment protocol (94-H-0010) for consideration for standard therapy or evaluation for eligibility for another branch protocol, depending on what is considered to be in the best interest of the subject.

9 DATA AND SAFETY MONITORING

9.1 Safety Monitoring

Principal investigator: Accrual, efficacy and safety data will be monitored by the PI.

NHLBI IRB*:* Accrual and safety data will be reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed consent form will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to the Title 45 CFR 46. This committee must approve all amendments to the protocol or informed consent, review all SAEs, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

9.2 Event Characterization and Reporting

Events include adverse events (AE), serious adverse events (SAE), protocol deviations (PD), unanticipated problems (UP), and non-compliance.

The principal investigator will review all events (AEs, protocol deviations, UPs, SAEs) to determine the seriousness, expectedness, and reportability of the event. As required and/or needed, the principal investigator will review the events with the Sponsor to make the final determination of seriousness and reportability.

9.2.1 Definitions

Adverse Event (AE): Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Serious Adverse Event (SAE): A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

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.

Serious event: An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

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.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets all of the following criteria::
1. 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
2. related or possibly related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. Serious non-compliance: Non-compliance that:
a. Increases risks, or causes harm, to participants.
b. Decreases potential benefits to participants.
c. Compromises the integrity of the NIH HRPP.
d. Invalidates the study data.

2. Continuing non-compliance: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. Minor (non-serious) non-compliance: Non-compliance that, is neither serious nor continuing.

9.2.2 Adverse Events Management

. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease. AEs will be graded by severity utilizing CTC version 2.0. A copy of the criteria can be down-loaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html.

Abnormal laboratory findings used to evaluate the safety of this protocol regimen will be collected to include any change from laboratory assessments done prior to first dose of study medication that result in a progression to a grade 3 or 4 laboratory toxicity. The laboratory toxicities will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease. They will be graded by severity utilizing CTC version 2.0.

Length of Adverse Event reporting: Adverse event collection will continue for 6 months after the last dose of Campath after which the events will be recorded in the medical record but not abstracted to toxicity tables. Serious adverse event reporting will continue as long as the subject remains on study.

9.2.3 Serious Adverse Events Management

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely (doubtfully related to the research) and unrelated (clear not related to the research).

Treatment related SAEs (TRSAEs) are those attributed as definitely, probably, or possibly. As detailed in section 8.5 stopping rules, only Death and any grade IV toxicities considered to be definitely related to alemtuzumab (Campath-1H®) will be monitored and considered for early stopping the study according to statistically determined criteria. John Tisdale, MD, NIDDK will serve as the independent monitor who reviews the attribution of TRSAEs

Hospitalization (overnight admission) for routine supportive care (platelet or RBC transfusions) or admission from the NIH inpatient unit to the NIH ICU for routine monitoring will not be reported as a serious adverse event.

9.2.4 Reporting Events

Principal Investigator*:* All events will be reported to Stefan Cordes, M.D. Principal Investigator of this study

Stefan Cordes, M.D. Bldg 10, CRC 4-5140
Phone: (301) - 402-2399
e-mail: stefan.cordes@nih.gov

9.2.4.1 Reporting Timeframes to IRB Chair, Clinical Director, and/or NHLBI IRB

Serious Events
Reports to the IRB and CD: The PI must report Serious UPs, and Serious PDs to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event using the NIH Problem Report Form.

Reports to the IRB Chair and CD: The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event, using the NIH Problem Form.

Non-serious Events
Reports to the IRB and CD: The PI must report all UPs that are not Serious to the IRB and CD, and PDs that are not Serious to the IRB, not more than 14 days after the PI first learns of the event using the NIH Problem Form.

Deaths
The PI must report all deaths (that are not UPs) to the CD as soon as possible, but not more than 7 days after the PI first learns of the event

9.2.4.2 At continuing review, the PI will provide to the IRB a summary of:

- All UPs
- All PDs
- All AEs (except for those granted a waiver of reporting)
- If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or investigational brochure (IB), these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above, and summarized at the time of continuing review.

Exclusions to data reporting:
The following Adverse Events will be captured only in the source documents and will not be reported to the IRB at the time of continuing review.
- Laboratory values that do not meet the definition of AE
- All grade 1 events listed as expected in the investigator's brochure, package insert or the protocol.

9.3 Data management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators, Hematology Branch fellows, research nurses and/or a contracted data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from home physician. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from referring home physicians will be entered into the system.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

Loss or destruction of data: Should we become aware that a major breech in the our plan to protect patient confidentiality and trial data has occurred, the IRB will be notified.

Publication Policy: Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional

approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP).

10 HUMAN SUBJECTS PROTECTION

10.1 Rationale for Subject Selection

This study will be open to subjects ages 18 and older who fit the inclusion criteria and provide informed consent to the protocol. As this is a rare disorder, distribution by race, gender or age cannot be predicted.

Recruitment: The study will be listed on clinicaltrials.gov, clinical center research studies, PDQ, Leukemia foundation and the NHLBI patient recruitment websites. If recruitment goals are not met, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment.

Reimbursement: Reimbursement for protocol travel, food, and lodging will be consistent with NHLBI DIR Travel and Lodging Compensation of Clinical Research Subjects policy or institutional guidelines.
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Payment for participation: $0

10.2 Participation of children.

In principle, age is not a consideration. But in practice we are limiting the protocol to subjects $\geq$ 18 years of age. T-large Granular Lymphocyte (T-LGL) Lymphoproliferative disorders largely afflict adults, therefore, given the rarity of these conditions in children as compared to adults, an extraordinary effort would be needed to include children, and it would be improbable that an adequate number of pediatric subjects could be accrued to statistically evaluate endpoints.

10.3 Risks and Discomforts

10.3.1 Related to alemtuzumab (Campath®)

Alemtuzumab (LEMTRADA previously marketed as Campath) may cause an increased risk of malignancies, including thyroid cancer, melanoma, and lymphoproliferative disorders in patients with multiple sclerosis (Product Label, approved on 11/14/2014).

The most commonly reported infusion related adverse events were rigors (86%), drug-related fever (85%), nausea (54%), vomiting (41%), and hypotension (32%). Hematologic toxicities included pancytopenia/marrow hypoplasia (6%), anemia (80%), thrombocytopenia (72%), neutropenia (85%), and profound lymphopenia, and should be monitored. Infections reported included sepsis (15%), pneumonia (16%), and opportunistic infections such as CMV (8%), Candidiasis (5%), Aspergillosis (2%), and Mucormycosis (2%).

Infusion-related symptoms and signs: Alemtuzumab can result in serious infusion reactions which include nausea, vomiting, fever, chills, rigors, hypotension, rash, fatigue, headache, diarrhea, pruritus, urticaria, bronchospasm and dyspnea. An attempt to prevent these reactions is made by using premedication. Subjects will be carefully monitored during infusions. In case of mild reaction, the subject will be treated according to symptoms (antiemetics, IV fluid hydration, acetaminophen, antihistamines,

inhaled bronchodilators). In case of moderate or severe reaction, the infusion will be discontinued and restarted at a lower rate once symptoms have subsided.

Infections: Serious, sometimes fatal bacterial, viral, fungal and protozoan infections have been reported in patients receiving alemtuzumab (Campath®). Subjects will be at risk for opportunistic infections. Therefore subjects will receive prophylaxis for Herpes virus and *Pneumocystis jiroveci.*

Cardiac toxicities: The following were reported in at least one patient treated on studies where alemtuzumab was used as a single agent: cardiac failure, cyanosis, atrial fibrillation, cardiac arrest, ventricular arrhythmia, ventricular tachycardia, angina pectoris, coronary artery disorder, myocardial infarction, and pericarditis. Some of these cardiac abnormalities may be irreversible. For this reason, we will monitor subjects with an echocardiogram and a 24 hour Holter monitor before treatment begins and after the last dose of alemtuzumab (Campath®). The ECHO will also be repeated at the 3 month follow up visit. We will closely monitor subjects for cardiac symptomology and ask them to immediately report any cardiac symptoms (palpitations, irregular pulse, difficulty in breathing, dizziness, swelling in the ankles, chest discomfort or pain).

Hematologic toxicities: Myelosuppression involves all 3 cell lineages. Serious and in rare instances fatal pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab (Campath®).

Other side effects (> 5% in CLL studies) include headache, anorexia, asthenia, skeletal pain, myalgias, peripheral edema, dysthesias, dizziness, tremor, stomatitis, mucositis, dyspnea, cough, pneumonitis, rhinitis, abdominal pain, back pain, dyspepsia, constipation, insomnia, depression, somnolence, diarrhea, and EBV related lymphoproliferative disease.

Pregnancy and breast feeding: Animal reproductive studies have not been conducted. It is not known whether alemtuzumab can affect reproductive capacity or cause fetal harm. Human IgG is known to pass the placental barrier and thus may potentially cause fetal B-cell depletion; therefore alemtuzumab should only be given to pregnant women only if clearly needed. It is not know whether alemtuzumab is excreted in human milk. Because human IgG is excreted in human mild and the potential for absorption and immunosuppression in the infant is unknown, women should be advised to discontinue nursing for at least 3 months following last dose of alemtuzumab (Campath®).

10.3.2 Related to bone marrow aspirate and biopsy: No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

10.3.3 Related to blood draws: No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, vasovagal reactions or infections may rarely occur.

10.3.4 Related to Cardiac monitoring

- ***EKG*:** An electrocardiogram (EKG) is a test that measures the electrical activity of the heartbeat. With each beat, an electrical impulse (or "wave") travels through the heart. This wave causes the muscle to squeeze and pump blood from the heart. A technician will put patches (electrodes) on the chest, arms and legs. The electrodes are soft and don't cause any discomfort when they're put on or taken off by the technician. The machine only records the EKG. It doesn't send electricity into the body. There's no pain or risk associated with having an electrocardiogram

- ***ECHO*:** The ECHO uses sound waves to visualize and evaluate the function of the heart. There are no associated risks

- ***Holter Monitor*:** The Holter involves wearing a monitor for 24 hours during which time the electrical activity of the heart is recorded. There are no associated risks other than the inconvenience of wearing the apparatus.

10.3.5 Related to central line placement: A catheter may be placed in a large vein of the neck, chest, or arm using local anesthetic. Subjects will sign a separate consent for the placement procedure. Only trained experienced staff will place the line in order to minimize these procedure related risks

The risks from the procedure are low; they include bleeding, bruising, or infection at the site of insertion. Very rarely (less than 1% of the time), the line placement may nick a vein causing one lung to collapse during line insertion. If the lung collapses, a tube may have to be inserted into the chest and remain in place until the lung re-expands. Because of this risk, subjects will have a chest x-ray following the procedure to make sure the line is in the correct place and that the lung is not collapsed. Once placed, the line will remain in place until drug administration is complete.

10.3.6 Related to Infection medications

- ***Pentamidine:*** cough (31-47%), bronchospasm (10-23%), decreased appetite (53-72%), fatigue, metallic taste, shortness of breath, decreased appetite, dizziness, rash, nausea, pharyngitis, chest pain/congestion, night sweats, chills, vomiting.

- ***Valacyclovir***: Nausea and/or vomiting, headache, dizziness, abdominal pain, dysmenorrhea, arthralgia, acute hypersensitivity reactions, elevations in liver enzyme laboratory values (e.g. AST). Renal failure and CNS symptoms have been reported in patients with renal impairment who received valacyclovir at greater than the recommended dose

- ***Cirpofloxacin*** (Cipro, Cipro XR, Proquin XR) The most frequent side effects include nausea, vomiting, diarrhea, abdominal pain, rash, headache, and restlessness. Rare allergic reactions have been described, such as hives and anaphylaxis (shock). Rare instances of seizures, other neurologic sequella and tendon rupture have been reported. Drug interactions with other medications have been reported and these will be reviewed before antibiotic start. July 8th 2008 the FDA warned the prescribing community of the newly required "black box warning" that caution patients over 60, those taking corticosteroids, and those who've undergone heart, lung, or kidney transplants are at increased risk of tendon rupture or tendinitis if they take fluoroquinolones. FDA estimates that spontaneous ruptures occur in about one in 100,000 people and that taking this class of drugs appears to triple or quadruple the risk. Most of the reported tendinitis and tendon ruptures have affected the Achilles tendon, behind the ankle. But the agency has also received reports of tendinitis and ruptures in the shoulder and hand.

10.3.7 Related to Apheresis

The apheresis procedures will be performed in accordance with standard apheresis donation policies and procedures operative in the Dept. of Transfusion Medicine and will be in compliance with the Blood Donor Standards of the American Association of Blood Banks and the rules and regulations of the Food and Drug Administration. Adverse reactions to apheresis procedures are rare, but include:

- Pain and hematoma at the needle placement site
- Vasovagal episodes, characterized by transient hypotension, dizziness, nausea and rarely, syncope are seen in less than 2% of the procedures. Hypotension secondary to volume depletion is known for the rare potential for a cerebrovascular or cardiovascular event.
- Cutaneous or circumoral parasthesias, chills, nausea, heartburn and rarely muscle spasms may result from the use of citrate anticoagulant used to prevent clotting in the extracorporeal circuit. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets or with intravenous calcium gluconate.

Prior to each apheresis, the potential risks associated with the procedure will be explained to the patient and a separate informed consent obtained.

10.4 Risks in relation to benefits

For adult subjects:

The potential benefit to the subject include reduction or even abolition of transfusion requirements and/or improvement of cytopenia, resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents, iron overload, and/or a susceptibility to infections. Potentially, treatment with other more toxic therapies could also be avoided or postponed.

Therefore, the research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CRF 46.102)

10.5 Informed Consent process and procedures

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the subject during the initial clinic evaluation. A copy of the consent form will be presented to the subject, with instructions to note down questions or underline areas of the consent form they do not properly comprehend. The Principal Investigator or an associate investigator on this protocol with an asterisk beside their name of the cover page will lead this discussion and obtain the informed consent. The consent form will be signed in the presence of the investigator and a witness prior to commencement of the treatment plan. The treatment plan and risks will be discussed again and in detail during their hospital visit for treatment.

If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective subject. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

Informed Consent of Non-English Speaking Research Participants:
If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved

consent document.

We request prospective IRB approval of the use of the short form for up to a maximum of 5 participants and we will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and that we will have that consent document translated into the given inherent language.

Informed Consent for adult research participants unable to provide consent:

If there is an unexpected enrollment of a research participant unable to provide informed consent, the following justification and procedures per NIH HRPP SOP 14E will be used to enrolled participants in the this protocol.

Justification for inclusion: This research provides the prospect of direct benefit, therefore inclusion is justified. The benefits to the participants could be reduction or even abolition of transfusion requirements and/or improvement of cytopenias resulting in improved quality of life and also decreased morbidity and mortality from transfusion-associated viral agents. Potentially, bleeding complications and treatment with other more toxic therapies could also be avoided or postponed. Not allowing participants who cannot provide consent would deny them the potential benefits this protocol offers for their MDS. There are no plans to include institutionalized participants.

Risk/Benefit Assessment:

This research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102)

Consent and Assent:

Procedures to determine capacity: If documentation of decision making capacity is not present in the medical record or the investigator questions the decision making capacity of the individual, then the Ability to Consent Assessment Team (ACAT) (301-496-9675 or 301-496-2429) will be contacted to make the determination.

Procedures for obtaining consent for legally authorized representative (LAR) (Risk Level B per SOP 14E): The following procedures will be followed starting with (1) in order to determining the LAR.

(1) For adults who cannot consent and have a court appointed guardian from a jurisdiction that allows it or a Durable Power of Attorney (DPA) for health care and/or research participation, the PI/designee or ACAT confirms appropriateness of surrogate to consent to research, including that:
(a)The surrogate understands that the protocol involves research;
(b)The surrogate understands the risks, potential benefits, (if any), and alternatives to the study;
(c)The surrogate has sufficient reason to believe participation in the study is consistent with the subject's preferences and values

(2) Adults who cannot consent and who do not have a DPA or court-appointed guardian, but who are capable of understanding the DPA process and can assign a DPA, then ACAT confirms appropriateness of surrogate to consent to research, which includes assessing criteria (a)-(c) above.

(3) Adults who cannot consent, who do not have a DPA or court-appointed guardian, and who are not able to understand the DPA process to appoint a DPA, then a person at the highest level of the following list may serve as surrogate and authorize subject's

participation if ACAT confirms surrogate appropriateness (which includes assessing criteria (a)-(c) above):
1. spouse or domestic partner;
2. adult child;
3. parent;
4. sibling;
5. other close relative

If at any time there is a question about the authority of the LAR to provide consent based on the jurisdiction appointing the durable power of attorney or other legal question regarding the LAR to provide consent, the Office of the General Counsel will be consulted.

Procedures to obtain assent and documentation of assent or dissent: The informed consent discussion will include the individual unable to provide informed consent along with LAR. The individual unable to provided informed consent will be asked if they agree to participate in the research and this will be documented in the medical record.

10.6 Conflict of Interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team reported a potential conflict of interest.

11 ACKNOWLEDGEMENT

We would like to acknowledge the contribution of Pierre Noel, M.D. in providing his expertise in LGL towards the development of this research protocol.

12 PHARMACEUTICALS

12.1 ALEMTUZUMAB (CAMPATH®)

Generic: alemtuzumab, **NSC**#715959
Classification: monoclonal antibody
Action: Monoclonal antibody directed against CD52 antigen, a surface glycoprotein expressed by lymphocytes
Pharmacokinetics: Campath pharmacokinetics were characterized in a study of30 Campath-naïve patients with chronic lymphocytic leukemia(B-CLL) who had failed previous therapy with purine analogs. Campath was administered as a 2 hour intravenous infusion, at the recommended dosing schedule, starting at 3 mg and increasing to 30 mg three times per week for up to 12 weeks. Campath pharmacokinetics displayed nonlinear elimination kinetics. After the last 30 mg dose, the mean volume of distribution at steady-state was 0.18 L/kg (range: 0.1 to 0.4 L/kg).Systemic clearance decreased with repeated administration due to decreased receptor-mediated clearance (i.e., loss of CD52receptors in the periphery). After 12 weeks of dosing, patients exhibited a seven-fold increase in mean AUC. Mean half-life was11 hours (range: 2 to 32 hours) after the first 30 mg dose and was 6 days (range: 1 to 14 days) after the last 30 mg dose. Comparisons of AUC in

patients 65 years or older (n=6) versus patients less than 65 years (n=15) suggested that no dose adjustments are necessary for age. Comparisons of AUC in female patients (n=4) versus male patients (n=17) suggested that no dose adjustments are necessary for gender. The pharmacokinetics of Campath in pediatric patients have not been studied. The effects of renal or hepatic impairment on the pharmacokinetics of Campath have not been studied.

Availability/Supply: Available through the Campath Distribution Program (The Sanofi Foundation for North America 1-877-422-6728). Vials are provided through this program upon completion of a patient specific request form. Prior to submission of a drug request the patient must provide authorization for the release of medical information (NIH-527). Refer to the Pharmacy Department or Clinical Pharmacy Specialist for additional details on drug procurement. Each single use, clear glass vial of Campath contains 30 mg Alemtuzumab in 1 mL of solution (8.0 mg sodium chloride, 1.44 mg dibasic sodium phosphate, 0.2 mg potassium chloride, 0.2 mg monobasic potassium phosphate, 0.1 mg polysorbate 80, and .0187 mg disodium edetate dehydrate). No preservatives are added. Each carton contains three Campath vials (NDC 50419-357-03) or one Campath vial NDC 50419-357-01).

Product description: Alemtuzumab injection is available in single-use, clear glass vials containing 30 mg of alemtuzumab in 1 ml of solution

Storage: Stored at 2 to 8 degrees Celsius (36 to 46 degrees Fahrenheit) and protected from direct sunlight. Protect from freezing; discard if frozen.

S*tability*: Diluted solution for administration can be stored at room temperature (15 to 30 degrees Celsius) or refrigerated, and should be used within 8 hours after dilution; protect solution from light.

Preparation for Administration: Parenteral drug products should be inspected for visible particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. **DO NOT SHAKE VIAL PRIOR TO USE.** As with all parenteral drug products, aseptic technique should be used during the preparation and administration of alemtuzumab (Campath®). Withdraw the necessary amount of alemtuzumab from the vial (solution concentrated to 30mg/ml) into a 1 ml syringe calibrated in increments of 0.1 ml. Inject into 100 mL sterile 0.9% Sodium Chloride USP or 5% Dextrose in Water USP. **Gently invert the bag to mix the solution.** Discard syringe and any unused drug product.

12.2 CIPROFLOXACIN

Drug Class and Mechanism: Many common infections in humans are caused by single cell organisms, called bacteria. Bacteria can grow and multiply, infecting different parts of the body. Medicines that control and eradicate these bacteria are called antibiotics. Ciprofloxacin is an antibiotic that stops multiplication of bacteria by inhibiting the reproduction and repair of their genetic material (DNA).

Supply: Commercially available

Drug interactions: Ciprofloxacin administered together with theophylline can lead to elevated blood levels of theophylline. Theophylline is used to open airways in the treatment of asthma. Toxic levels of theophylline can lead to seizures, and disturbances in heart rhythm. If concurrent use of ciprofloxacin and theophylline cannot be avoided, frequent blood tests to monitor theophylline blood levels are performed. Ciprofloxacin should be used with caution in subjects with central nervous system diseases such as seizures, because rare seizures have been reported in subjects receiving this medication. Many antibiotics, including ciprofloxacin, can alter the normal bacteria in the colon and encourage overgrowth of a bacteria responsible for the development of inflammation of the colon (pseudomembranous colitis). Pseudomembranous colitis can cause fever, abdominal pain, diarrhea, and sometimes even shock. Patients taking ciprofloxacin can develop sensitivity of the skin to direct sunlight. Ciprofloxacin can enhance the action of the anticoagulant warfarin (Coumadin), and increase the risk of bleeding.

Storage and Stability: Ciprofloxacin should be stored at below 86 degrees F

Administration: Ciprofloxacin may be taken with or without food. Ciprofloxacin is partially metabolized by the liver and excreted by the kidney. Dosages require adjustment in patients with severely abnormal liver or kidney function. Antacids block the absorption of ciprofloxacin and they should not be taken together.

*Adverse reaction*s: see section 10.3.8, Risks and discomforts

12.3 PENTAMIDINE

Supply: Commercially available (NebuPent®, American Pharmaceutical Partners, Inc.)

Product description: Pentamidine isethionate is available as a 300 mg single dose vial containing 300 mg of lyophilized powder in a 15 mL capacity vial. The contents of one vial must be dissolved in 6 mL of sterile water for injection, USP. It is important to use only sterile water; saline solution will cause the drug to precipitate.

Storage and stability: Store dry product at controlled room temperature 15-30°C (59-86°F).

Route of administration: Inhalation; Once reconstituted, the entire contents of a vial should be placed into the Respigard® II nebulizer (Marquest) reservoir for administration by inhalation Do not mix the pentamidine solution with any other drugs.

12.4 VALACYCLOVIR

Generic name: valacyclovir

Brand Name: Valtrex

Supply: Commercially available.

Pharmacology: Valacyclovir is the hydrochloride salt of L-valyl ester of the antiviral drug acyclovir. After oral administration, valacyclovir is rapidly absorbed from the GI tract and nearly completely converted to acyclovir and L-valine by first-pass intestinal or hepatic metabolism.

Product description: Valacyclovir is available in 500mg tablets and 1gm tablets. Dose adjustment is necessary in patients with significant renal impairment (refer to the manufacturer's labeling for dose adjustment guidelines.

Storage and Stability: Oral tablets should be stored at 15° to 25°C (59° to 77°F).

Route of administration: Oral

APPENDIX A NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES

NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES v. 2/5/2013

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	**Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?**	**Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?**
A	**Stem Cell Allotransplantation Section (Dr. A. John Barrett)**		
A.1	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
A.2	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
A.3	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
A.4	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies.	No	No
A.5	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
A.6	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
A.7	Identification of individual T cell clones by their T cell receptor sequence.	No	No
A.8	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA,protein, or peptide expression in cells or fluids.	No	No
A.9	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No
A.10	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
A.11	Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions.	No	No
B	**Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)**		
B.1	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No

B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No
C.5	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
C.6	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
C.7	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
C.8	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
C.9	Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No

C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No
C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2.*	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No

E.10	VHL mutation analysis on kidney cancer tissue.	No	No
E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Lasar capture microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No

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