

CLINICAL RESEARCH PROJECT

Protocol #02-H-0250

Date: December 28, 2017

Title: A phase I/II study of HLA-matched mobilized peripheral blood hematopoietic stem cell transplantation for advanced mycosis fungoides/Sezary syndrome using nonmyeloablative conditioning with Campath-1H

Other underlying words: Cutaneous T Cell Lymphomas, Allogeneic PBSCT, Low intensity

Principal Investigator:

*Georg Aue, M.D., HB, NHLBI (E) 451-7141 Bldg 10, CRC 3-3216

Medically Responsible Investigator:

*Richard W. Childs, M.D., Clinical Director NHLBI, HB, NHLBI (E) 594-8008 Bldg 10, CRC 3-5332

Associate Investigators:

Lisa Cook, R.N., Research Nurse HB, NHLBI (E)	402-5609	Bldg 10, CRC 3-3485
Nancy Geller, Ph.D., Director, Office of Biostatistics, NHLBI (E)	435-0434	RKLG RM 9202
Xin Tian, Ph.D., Biostatistician, NHLBI (E)	435-1298	RKLG RM 9208
Brian Wells, PA-C, HB, NHLBI (E)	451-7128	Bldg 10, CRC 3-1341
Tatyana Worthy, RN, HB, NHLBI (E)	594-8013	Bldg 10, CRC 3-3485
*Ramaprasad Srinivasan, M.D., Ph.D., UOB, NCI (E)	496-6353	Bldg 10, CRC 2-5950
Geri Hawks, RN, NHLBI (E)	451-1173	Bldg 10, CRC 3-3485

Estimated Duration of Study: 15 years

Subjects of study	Number	Sex	Age range (years)
Recipients	Up to 25	either	18-70 (both inclusive)
Donors	Up to 25	either	18 and older
Total accrual	Up to 50		

Project involves ionizing radiation? Yes (medically indicated only)

Off site project? No

Multi-Institutional project? No

DSMB involvement? Yes

Tech Transfer Yes

* *Investigators who can obtain consent*

PRECIS

Primary cutaneous T-cell lymphomas (CTCL) are a group of lymphoproliferative disorders characterized by localization of neoplastic T cells to the skin at presentation. Mycosis fungoides (MF) and its leukemic variant Sezary syndrome (SS) are the most prevalent forms of CTCL. While early stage MF is restricted to patches and plaques involving the skin, most patients eventually develop cutaneous tumors, generalized erythroderma, or dissemination to peripheral blood, lymph nodes or visceral organs. Currently existing therapy of tumor-stage and disseminated CTCL is palliative, with most patients dying within 1-5 years. The presence of CD8+ cells in close proximity to dermal neoplastic infiltrates in early stages of the disease, and the clinical response seen with some immunomodulatory agents suggests that CTCL may be potential targets for immunotherapy-based interventions.

Allogeneic stem cell transplantation is a curative treatment modality successfully employed in a number of hematologic malignancies. The curative effect of this approach is in part mediated by donor-derived T lymphocytes with reactivity for patient leukemic cells. The power of this graft versus leukemia effect (GVL) is best illustrated in patients with relapsed Chronic Myeloid Leukemia (CML) after an allogeneic bone marrow transplant, in whom a single donor lymphocyte infusion (DLI) can induce remission. We hypothesize that neoplastic T cells in MF/SS may similarly be susceptible to a graft vs. tumor (GVT) effect. Unfortunately, advanced patient age and a 25% to 35% risk of transplant related mortality (TRM) preclude the use of conventional “dose- intensive” allogeneic peripheral blood stem cell transplantation (PBSCT) in patients with advanced CTCL who might otherwise benefit from this approach. The risk of TRM related to conditioning can be circumvented at least partially by using a reduced-intensity conditioning regimen to prepare the patient for transplantation.

In this study, we will treat male and non-pregnant female subjects between the ages of 18 and 70 years (both inclusive) suffering from advanced MF/SS with an allogeneic peripheral blood stem cell (PBSC) transplant from an HLA-matched family donor or an HLA matched (10/10 allele level match) unrelated donor. A low intensity, nonmyeloablative conditioning regimen employing the anti-CD52 monoclonal antibody Campath-1H (alemtuzumab) and fludarabine will be used to induce host immunosuppression to facilitate donor hematopoietic and lymphoid engraftment. We anticipate minimal host myelosuppression and consequently reduced early transplant toxicity with this conditioning regimen. Immune and hematopoietic reconstitution will be achieved by infusion of unmanipulated donor-derived granulocyte colony stimulation factor (G-CSF) mobilized peripheral blood stem cells. Infusions of donor lymphocytes in incremental doses will be used to promote engraftment or disease regression when indicated. Cyclosporine A (CSA) will be used as prophylaxis against graft vs. host disease (GVHD), with dose adjustments made as necessary to favor complete donor chimerism and disease regression.

A total of up to 25 subjects (transplant recipients) will be treated on this protocol. The primary end point of this study is efficacy (proportion of subjects achieving a complete response). Other end points include assessment of donor-host chimerism in various hematopoietic and lymphoid cells, overall response, incidence of acute and chronic GVHD, graft failure, assessment of lymphoid subset reconstitution, transplant related morbidity and mortality and disease-free and overall survival and the outcomes of transplantation by the donor type (related vs. unrelated).

TABLE OF CONTENTS

PRECIS	2
1. OBJECTIVES	4
2. BACKGROUND	4
3. STUDY DESIGN.....	8
4. SCIENTIFIC AND CLINICAL JUSTIFICATION.....	9
5. ELIGIBILITY ASSESSMENT	9
6. CLINICAL EVALUATION OF RECIPIENT	11
7 TREATMENT PLAN - RECIPIENT	13
8 DONOR EVALUATION AND PLAN	16
9 MANAGEMENT OF RECIPIENT COMPLICATIONS.....	19
10 STUDY MODIFICATIONS.....	20
11 RESPONSE CRITERIA	20
12 ANCILLARY LABORATORY RESEARCH STUDIES.....	21
13 BIostatistical CONSIDERATIONS.....	22
14 REMOVAL FROM THE STUDY	24
15 DATA SAFETY AND MONITORING PLAN	25
16 HUMAN SUBJECT PROTECTIONS	28
17 PHARMACEUTICALS	34
18 REFERENCES	36
19 TABLES 1 and 2.....	41
APPENDIX A TNM CLASSIFICATION OF CUTANEOUS T-CELL LYMPHOMA	42
APPENDIX C PREPARATIVE REGIMEN.....	43
APPENDIX D DONOR STEM CELL MOBILIZATION, AND APHERESIS.....	44
APPENDIX E NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES.....	45

SEE ALSO Supportive Care Guidelines For Allogeneic Hematopoietic Stem Cell Transplant Recipients
<http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml>

IRB approved 10/21/2003, 6/2006 and 1/2011

1. OBJECTIVES

- 1.1 To evaluate the efficacy of nonmyeloablative PBSC transplant using Campath-1H (alemtuzumab)/fludarabine based conditioning in subjects with advanced mycosis fungoides (MF)/Sezary syndrome (SS)
- 1.2 To identify allogeneic graft versus tumor (GVT) effects in subjects with advanced MF/SS following a nonmyeloablative allogeneic PBSC transplant
- 1.3 To evaluate the engraftment rate, safety and toxicity of alemtuzumab based nonmyeloablative preparative regimen using an HLA matched family or matched unrelated donor (10/10 allele level match) allogeneic blood stem cell transplant in subjects with MF/SS.
- 1.4 To evaluate overall response, disease free survival and overall survival after the transplant
- 1.5 To evaluate the rate and extent of lymphocyte subset reconstitution
- 1.6 To evaluate transplant related morbidity, including rates of acute and chronic GVHD and infectious complications, and mortality related to the transplant.
- 1.7 To evaluate the outcomes of transplantation by the donor type (related vs. unrelated).

2. BACKGROUND

2.1 Introduction

Although MF/SS is a relatively rare neoplasm, the disease appears to be on the increase, with a 3.2 fold increase being observed between 1973 and 1984 [1-3]. The majority of the patients are between 40 and 60 years of age and the disease is more common in males (M: F =2.2:1) and African Americans.

The disease often presents as an indolent dermatologic affliction which may go unrecognized for years. Characteristically, MF evolves through several clinical stages. The earliest stage is the patch phase typified by the presence of scaly, erythematous patches, which evolve into plaques and then into ulcerated or exophytic tumors with progressively more neoplastic infiltration. Sezary syndrome is characterized by the presence of large, atypical lymphocytes with cerebriform nuclei in the peripheral circulation, often accompanied by generalized erythroderma. Extracutaneous involvement becomes evident as the stage or extent of MF increases, with lymph nodes, spleen, lung and liver being involved most often. Bone marrow involvement occurs in later stages, being less common than visceral and lymph node involvement.

The malignant cell in MF/SS is of T-cell origin [4]. MF cells characteristically express the pan-T cell markers CD2, CD3 and CD5 [5-9]. The early T cell marker, CD7 is present in a third of cases [10]. The vast majority of the malignant cells are of the CD45+RO+CD4+ phenotype [8-11]. These cells also express a skin homing signal called cutaneous lymphoid antigen (CLA) [12]. Approximately 50% of cases express CD25 [13], an observation that has led to the evaluation of targeted immunotherapeutic approaches against this antigen. Expression of CD52 (the target of the monoclonal antibody Campath) by the malignant cells has not been widely studied, but 7/7 patients with SS expressed CD52 in one analysis [14] as did 3/3 patients in another analysis [15].

The prognosis of MF patients is stage-dependent (Section 19, Tables 1 and 2), with stage Ia patients exhibiting virtually normal life expectancies [16, 17]. Patients who progress to the tumor or erythrodermic phase, and those with lymph node or peripheral blood involvement (SS), or visceral metastases, however, are considered incurable. These patients often undergo a series of palliative treatments, eventually dying from their disease or related complications [16-18]. Five-year survival rates for stages IIb to IVb range from 10-50% [1,2,17,18], and in one series of 152 patients, those with visceral disease or lymph node effacement had a median survival of 2.5 years [19].

2.2 Current therapies for MF/SS

2.2.1 Skin Directed Therapy

Topical Chemotherapy with either mechlorethamine or carmustine (BCNU) is effective in controlling disease in patch or plaque- stage disease. Overall response rates of 88% and complete response (CR) rates of 51% for T1 disease and 70% overall response and 26% CR rates for T2 disease was reported in one series of patients by Hoppe et al [20]. This therapy is relatively safe and results in long-term remissions in 10-15% of patients with early stage disease [21].

Total Skin Electron Beam Therapy (TSEBT) results in a 56% to 96% CR rate in patients with stage Ia to IIa disease. However, relapse free survival for stage Ib and above is only 16% at 10 years [22, 23]. TSEBT is also an excellent palliative option for patients with diffuse involvement with thick plaques or tumors and for those with generalized erythroderma. In patients with T3 or T4 disease, disease free survival and overall survival at 5 years is 20% and 50% respectively [24]. Use of adjuvant systemic chemotherapy after TSEBT appears to prolong disease-free survival (DFS) without impacting on overall survival [25]. However, a preliminary study of the use of photopheresis in an adjuvant setting in T3 and T4 patients achieving a CR with TSEBT has shown promise and is being further investigated [26,27].

PUVA therapy consists of ingestion of 8-methoxypsoralen followed by exposure to UV radiation. Stage-dependant response rates of 60% to 95% have been reported with this therapy [28].

2.2.2 Systemic Therapies

Systemic Chemotherapy is used as palliation for patients with relapsed or refractory disease following treatment with skin directed therapies or for patients with advanced disease at presentation. Single agent therapy with several agents including methotrexate, glucocorticoids, nitrogen mustard, cyclophosphamide, cisplatin and the purine analogs (2-deoxycoformycin, 2-chlorodeoxyadenosine, and fludarabine) has been studied. Short lived remissions ranging from 30% to 50% have been reported with these agents [29-33].

The Interferons: Alpha interferon has been used with fair success. In previously untreated patients, doses between 3,000,000 units/day to 15,000,000 units/day have yielded response rates of around 80% [34, 35]. In contrast, previously treated patients showed objective response rates of 45-64%, although these responses were short-lived (mean durations of 4.5 to 5.5 months) [35]. Other cytokines such as IL-2 have resulted in short-lived responses in small case series [2].

Retinoids are vitamin A derivatives with antiproliferative and differentiating effects in several malignancies. Molin et al have reported on a series of 28 patients with MF/SS undergoing therapy with oral retinoids; a response rate of 68% was observed, although most responses were of short duration [36].

Ontak is a fusion protein that combines active components of the diphtheria toxin with recombinant IL-2, thus targeting cells expressing CD25. A phase III trial of this drug in heavily pretreated patients with CTCL demonstrated an overall response rate of 30%, with a median duration of response of around 7 months [37].

Photopheresis involves ingestion of 8 methoxypsoralen, followed by isolation and UVA radiation of the mononuclear fraction, and is believed to act by both direct toxic effects on neoplastic T cells and by inducing cytotoxic T cells against malignant T cells via stimulation of antigen presenting cells. Edelson et al. [38] reported a 73% response rate using this approach in patients refractory to previous therapies. This cohort of patients, consisting largely of T4 patients with SS, has a reported median survival of 60 months, compared with a median survival of 30 months in stage-matched historic controls. This study, however, did not require genotypic evidence of clonality in peripheral blood T cells to make a diagnosis of SS; in fact, when this issue was addressed in a retrospective comparison of patients treated with photopheresis vs untreated controls, the survival advantage vanished [39]. This modality is currently being investigated in combination with chemotherapy.

Alemtuzumab has been used in the treatment of both advanced MF and the Sezary syndrome. Several recent reports detail the results in small groups of patients treated with this monoclonal antibody. Foukanelli et al. [40] treated three patients with SS resistant to prior single agent chemotherapy, all of whom experienced clinical responses (2CRs and 1PR) of varying durations (3-27 months). Ferrajoli et al [41] treated 68 patients with refractory hematologic malignancies expressing CD52. 2/6 CTCL patients treated in this study were reported to have undergone a partial remission. A third study, by Lundin et al [42], reported that 4 of 8 patients with MF/SS showed disease regression (2 cutaneous CRs and 2 PRs). Based on these data, alemtuzumab appears to have clinical efficacy in MF/SS, although larger studies are required to define its role in the management of these malignancies.

Cyclosporine A (CSA): The anti-proliferative effects of cyclosporine on T-cells make it an attractive option in T-cell malignancies. However, in clinical studies of refractory MF/SS patients, less than a third obtained a partial remission [43-45].

In summary, although several skin-directed and systemic therapies exist for MF/SS, these are largely palliative and have little impact on survival of patients with advanced CTCL.

2.3 MF/SS as a potential target for immunotherapy

Several lines of evidence suggest that immune mechanisms may play a significant role in the control of MF/SS. First, the presence of CD8+ T cells in epidermal infiltrates in close proximity to neoplastic T cells suggests that these tumor infiltrating T cells may contribute to immune surveillance against the tumor [46]. Consistent with this hypothesis are the observations that the circulating CD4+/CD8+ ratio is often inverted with advancing disease, particularly in those patients with SS, and that the proportion of tumor-infiltrating CD8+ cells diminishes with progression of cutaneous disease [47]. The observation by Hoppe et al. [46] that within each T stage, survival correlates positively with the proportion of infiltrating T cells lends further credence to the hypothesis that immune mechanisms may play a critical role in disease progression. Second, a study of Langerhans' cells by Meissner et al. [48] revealed that increased density of these potent antigen-presenting cells in tumor infiltrates

correlated with a better prognosis. Third, cytokine therapies used to enhance cellular immune responses have yielded clinical responses, albeit short-lived, in 40-80% of CTCL patients [49]. Lastly, recent case reports of clinical remission of refractory CTCL following allogeneic stem cell transplantation provide evidence for the existence of a graft vs. CTCL effect [50-52]. The most impressive of these reports details 5 patients with refractory CTCL (4 patients with SS and 1 patient with MF), all of whom achieved a CR after undergoing an allogeneic PBSC transplant from 6/6 HLA-matched sibling (4/5) or unrelated (1/5) donors [52].

2.4 Allogeneic stem cell transplantation as immunotherapy

The potent antitumor effects associated with allogeneic stem cell transplantation are largely due to the generation of graft vs. tumor effects mediated by host T cells. The existence of a graft vs. malignancy effect was first elucidated in patients with leukemias who were cured after an allogeneic transplantation, and is supported by several lines of evidence: 1) The observation that patients developing moderate to severe GVHD had lower relapse rates than those who did not develop GVHD [53,54] 2) Higher rates of leukemic relapse in CML patients receiving T-cell depleted as opposed to T-cell replete grafts [54-57] 3) Higher rates of CML relapse in recipients of syngeneic transplants when compared with those receiving transplants from HLA-matched non-twin siblings [54] 4) Complete and durable PCR remission of relapsed CML in 70-80% of patients treated with a single, unmanipulated, donor lymphocyte infusion [58] 5) Complete remission of EBV-associated lymphoproliferative disorder with a single infusion of donor derived lymphocytes [59,60].

These observations are further strengthened by durable clinical remissions seen in patients with other malignancies, including non-Hodgkin's lymphoma and multiple myeloma, who undergo allogeneic bone marrow transplantation (BMT) [61-65]. An underlying alloimmune mechanism is inferred in these patients from temporal relations between immune manipulations (such as withdrawal of cyclosporine, donor lymphocyte infusions) and disease regression. More recently, non-myeloablative peripheral blood stem cell transplants, which utilize allogeneic graft vs. tumor effects rather than high dose chemotherapy as the basis for tumor eradication, have demonstrated success in both hematologic [66-68] and solid malignancies [69-71]. Our recent study showing disease regression in patients with metastatic renal cell carcinoma following nonmyeloablative PBSCT amply supports the arguments favoring the existence of a graft vs. solid tumor effect [69]. Patients in this study had responses associated with withdrawal of cyclosporine, donor lymphocyte infusions, acute graft vs. host disease, and other immune manipulations such as treatment with α -interferon, IL-2 and GM-CSF (unpublished data). Laboratory data suggest that these responses are mediated at least in part by CD8 lymphocytes derived from the donor and directed against shared minor histocompatibility antigens and/or against tumor specific antigens.

Most updated data from Center for International Blood and Marrow Transplant Research (CIBMTR) and European Society for Blood and Marrow Transplantation (EBMT) provided a clearer picture of the value of allogeneic HCT as a therapeutic strategy for high-risk patients with advanced-stage MF/SS [99-100]. In particular, a large CIBMTR series with 129 cases of MF/SS reported an OS of 44% 2 years after HCT [99]. This large series of allogeneic HCT in MF/SS also confirmed feasibility, acceptable NRM (19–28%) and evidence of benefit in an advanced cohort of MF/SS patients. EBMT data showed that patients with advanced-stage MF/SS continue to benefit from allogeneic HCT over time, with an OS of 46% at 5 years and 44% at 7 years, and a PFS of 32% at 5 years and 30% at 7 years [100].

Recent data from large clinical trials have shown outcomes for patients suffering from a variety of different diseases (malignant and non-malignant) undergoing transplantation from HLA matched

unrelated donors molecularly matched at 10/10 alleles are similar to those of patients receiving a transplant from an HLA matched relative [93-95]. Retrospective data from 1997 to 2007 have shown transplants using unrelated donors can cure patients with MF/SS, although there was a statistically borderline increase in NRM and a lower PFS and OS [99, 100]. However, no prospective data describing outcome differences between the 2 groups based on related vs unrelated donors currently exists in the modern era where matched unrelated donors are typed using high resolution molecular typing to assure a perfect match at 10/10 alleles. There is also no current data comparing remission achievement in MF/SS patients who had a matched related donor versus a matched unrelated donor allogeneic transplant. Duarte et al [96] reported a total of only 21 MF/SS patients, 11 men and 10 women, from 1994 to 2008. Fourteen of these patients had advanced MF (stage IIB in 2, stage III in 1, stage IVA in 10, stage IVB in 1), 5 had SS and 2 others had high-grade lymphoma transformed from MF. Patients were very heavily pretreated, with a median of seven lines of treatment prior to allogeneic HSCT. Sixteen donors were HLA-identical siblings and five were matched unrelated donors. 15 out of 16 patients achieved CR and 1 achieved PR with matched related donor transplantation. All 5 patients who underwent matched unrelated transplantation achieved a complete clinical remission. Detailed data describing the outcomes of a cohort of 8 of the above mentioned MF/SS patients were published by Molina et al. [97], including 4 patients who underwent an HCT from a matched related donor and 4 patients who received a transplant from unrelated donors (4 patients). All patients achieved complete clinical remission and resolution of molecular and cytogenetic markers of disease within 30–60 days after HCT. Two patients died from transplantation-related complications. A comparison of matched related vs matched unrelated donor groups was not done in this study due to small patient numbers. With a median follow-up of 56 months, six patients remain alive and without evidence of lymphoma. The results suggest that allogeneic HSCT from both HLA-matched siblings and unrelated donors can induce durable clinical remissions in patients with advanced cutaneous T-cell lymphoma that is refractory to standard therapies.

2.5 **Campath -1H (Alemtuzumab)**

Alemtuzumab is a humanized rat monoclonal immunoglobulin (Ig) G1 directed against CD52 [72]. CD52 is a heavily glycosylated glycoprotein of molecular weight 21-28kD and in humans is expressed in over 95% of peripheral blood lymphocytes, monocytes and macrophages [73, 74]. A small proportion of NK cells, and neutrophils express CD52, but the antigen is absent from hematopoietic stem cells [15]. The activity of alemtuzumab derives from its ability to induce cell lysis by complement fixation and antibody-dependent cytotoxicity, upon engagement of its cognate antigen [75]. A variety of clinical applications have been studied in conditions ranging from autoimmune disorders, to B-CLL, T-CLL, low grade lymphomas, as well as in allograft T cell depletion for stem cell transplantation [42, 76-84]. The major toxicities of alemtuzumab are infusion-related adverse events, and infectious complications stemming from the profound immunosuppression induced by the drug [85].

Alemtuzumab is a potentially useful drug in nonmyeloablative conditioning, given its profound lymphotoxicity but relatively minor effects on cells of myeloid lineage. A recent study by Kottaridis et al. employed alemtuzumab as part of a nonmyeloablative conditioning regimen; patients in this study had a high rate of engraftment and very low incidence of severe GVHD [84]. Alemtuzumab also has activity against MF/SS, as demonstrated in several recent clinical trials [40-42].

3. **STUDY DESIGN**

3.1 **Peripheral blood hematopoietic progenitor cell transplant (PBPC)**

02-H-0250
Amendment V
Georg Aue, M.D.
December 28, 2017

T cell replete PBPC allograft: Subjects will receive a nonmyeloablative preparative regimen of alemtuzumab 30mg iv three times a week for two weeks followed by fludarabine 25mg/m²/day for five days followed by a PBPC graft targeted to deliver $\geq 5 \times 10^6$ CD34+ cells/kg. Cyclosporine A (CSA) for GVHD prophylaxis will be used initially with target CSA levels in the therapeutic range (200 -400 ng/ml).

The HLA matched related or unrelated donor (10/10 allele level match) will receive G-CSF as indicated in Section 8.3 with an apheresis collection of PBSC on day 5. G-CSF may be continued and additional leukapheresis performed after day 5 if required as outlined in Section 8.3.

3.2 Donor lymphocyte infusions (DLI) and cyclosporine A

On day 30, and subsequently, extent of donor T-cell chimerism will be assessed. On day +30, +60, and +100 disease status will be assessed in all recipients by clinical and radiographic examination. CSA management and DLI administration will follow the scheme outlined in section 7.10.

4. SCIENTIFIC AND CLINICAL JUSTIFICATION

This study will evaluate the efficacy of alemtuzumab and fludarabine based nonmyeloablative PBSC transplantation in subjects with advanced MF/SS. The study will also evaluate engraftment, safety and toxicity of this transplant approach.

Advanced MF/SS is an incurable disease and progression to tumor or erythrodermic phase, as well as presence of extracutaneous disease carries a poor prognosis.

MF/SS is amenable to immunotherapy as demonstrated by clinical remissions induced by cytokine therapy and by allogeneic stem cell transplantation.

Sufficient evidence exists to indicate that a graft versus tumor effect can be generated against a variety of malignant diseases following transplants utilizing low-intensity conditioning, with the added benefit of reduced transplant-related toxicity and mortality.

Thus, the investigation of nonmyeloablative peripheral blood stem cell transplantation in advanced MF/SS is justified. The subjects selected for the protocol will only include those for whom no reasonable curative alternative exists. The conditioning regimen has resulted in complete engraftment accompanied by CRs in both subjects transplanted on the initial version of the protocol

5. ELIGIBILITY ASSESSMENT

5.1 Inclusion criteria- recipient

5.1.1 Ages 18-70 years (both inclusive)

5.1.2 Stages IIb to IVb patients with MF (Appendix A)(biopsy diagnostic or consistent with MF) who have progressed despite at least one treatment regimen and all patients with SS

AND

Anticipated median survival less than 5 years or debilitation as a result of their disease

- 5.1.3 Recovery from acute toxicity of prior treatment for MF/SS (to \leq grade 1 [CTCAE v3.0]) or stabilization of toxicity occurring from prior therapy for MF/SS
- 5.1.4 HIV negative
- 5.1.5 ECOG performance status of 1 or less
- 5.1.6 No major organ dysfunction precluding transplantation
- 5.1.7 DLCO \geq 60% predicted
- 5.1.8 Left ventricular ejection fraction \geq 40%
- 5.1.9 Less than or equal to 25% of liver involved with metastatic tumor by CT scan
- 5.1.10 6/6 HLA matched family donor or 10/10 matched unrelated donor at the allelic level available
- 5.1.11 Ability to comprehend the investigational nature of the study and provide informed consent.

5.2 Inclusion criteria – related and unrelated donor

- 5.2.1 6/6 HLA- matched family donor or 10/10 HLA-matched unrelated donor
- 5.2.2 Age \geq 18 years
- 5.2.3 Ability to comprehend the investigational nature of the study and provide informed consent.
- 5.2.4 For unrelated donor, the NMDP unrelated donor inclusion criteria will be used as outlined in document (<http://bethematch.org/WorkArea/DownloadAsset.aspx?id=1960>). Donor eligibility will be completed per NMDP standards and in accordance with most recent and stringent FDA guidelines.

5.3 Exclusion criteria (any of the following)-recipient

- 5.3.1 Patient pregnant or lactating
- 5.3.2 Age >70 or <18 years
- 5.3.3 ECOG performance status of 2 or more (See supportive care guidelines for grading).
- 5.3.4 Psychiatric disorder or mental deficiency of the recipient or donor sufficiently severe as to make compliance with the BMT treatment unlikely and making informed consent impossible.
- 5.3.4 Major anticipated illness or organ failure incompatible with survival from BMT and where survival is considered insufficient to assess transplant outcome (i.e. < 3 months).
- 5.3.5 DLCO $< 60\%$ predicted
- 5.3.6 Left ventricular ejection fraction $< 40\%$
- 5.3.7 Serum creatinine > 2.0 mg/dl
- 5.3.8 Serum bilirubin >4 mg/dl, transaminases $>5x$ upper limit of normal
- 5.3.9 HIV positive
- 5.3.10 History of other malignancies in the last five years with the exception of basal cell or squamous cell carcinoma of the skin
- 5.3.11 Evidence for CNS metastatic disease
- 5.3.12 Disease involving $>25\%$ of the liver radiographically.

5.4 Exclusion criteria (any of the following) - related and unrelated donor

- 5.4.1 Donor pregnant or lactating
- 5.4.2 Age < 18 years
- 5.4.3 HIV positive (donors who are positive for hepatitis B (HBV), hepatitis C (HCV) or human T-cell lymphotropic virus (HTLV-1) will be used at the discretion of the investigator following counseling and approval from the recipient).
- 5.4.4 Sickling hemoglobinopathy including HbSS or HbSC (for unrelated donors, testing for hemoglobinopathies will only be done when clinically indicated).

- 5.4.5 History of malignancy within 5 years except basal cell or squamous carcinoma of the skin.
- 5.4.6 Donor unfit to receive G-CSF and undergo apheresis (uncontrolled hypertension, history of stroke, thrombocytopenia).
- 5.4.7 Psychiatric disorder or mental deficiency of the donor sufficiently severe as to make compliance with the BMT treatment unlikely and making informed consent impossible.

6. CLINICAL EVALUATION OF RECIPIENT

Bone marrow aspirates will be read by a pathologist. 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.

6.1 Pre-study evaluation

Pre-study evaluation tests will be conducted on a Hematology Branch screening protocol such as 97-H-0041. All pre-study evaluation tests must be done within 4 weeks of signing consent except for blood tests outlined below in Section 6.1.9 which must be done within 14 days (≤ 14 days) of signing consent.

- 6.1.1 Biopsy of accessible disease site, when possible, for confirmation of diagnosis, and identification of tumor infiltrating lymphocytes (TIL). Laparoscopy, thoracoscopy, or other invasive interventional procedures will be performed only to confirm the diagnosis of extracutaneous disease and not for solely research purposes
- 6.1.2 Photographic evaluation of skin lesions
- 6.1.3 MRI of the brain to rule out metastatic disease involving the CNS
- 6.1.4 Computerized tomography (CT) scans of the neck, sinuses, chest, abdomen, and pelvis
- 6.1.5 Complete history and physical exam with emphasis on documenting measurable lesions
- 6.1.6 HLA -A, -B, -DR molecular typing, ABO, Rh typing, RBC antibody screen
- 6.1.7 Bone marrow aspirate and biopsy (chromosome analysis, PCR (for T cell gene rearrangement when applicable), flow cytometry).
- 6.1.8 Type and antibody screen for HBV, HCV, HIV, HTLV-1, CMV, EBV, toxoplasma, and VZV serology
- 6.1.9 Coagulation profile, CBC with differential, - Acute Care (Na, K, Cl, CO₂, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LDH) panel, reticulocyte count, prealbumin,
- 6.1.10 Thyroid panel, FSH (only in females), LH (only in females), random cortisol level (as clinically indicated)
- 6.1.11 Iso-heme titers
- 6.1.12 Hemoglobin electrophoresis
- 6.1.13 Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) scan
- 6.1.14 Pulmonary function: vital capacity, FEV₁, DLCO
- 6.1.15 PPD placement
- 6.1.16 Serum troponin-I level
- 6.1.17 Cardiac function: EKG, 24 hour holter monitor, ECHO,. All subjects age ≥ 50 , or age ≥ 40 , with one or more risk factors for coronary artery disease (history of high blood pressure, increased cholesterol, smoking, or diabetes or family history of coronary disease), will have a baseline cardiac workup, which will include:
 - Cardiology consultation and further workup as deemed necessary by Cardiology consultant

- 6.1.18 Nutritional assessment (as clinically indicated)
- 6.1.19 Social work consult
- 6.1.20 Ophthalmology consult
- 6.1.21 Pregnancy test in women
- 6.1.22 Dermatology consult
- 6.1.23 Measurement of skin lesions when applicable

6.2 In-Patient/Outpatient Monitoring to Day 100

Once daily: (when in-patient, as clinically indicated) CBC with differential, Acute Care, Mineral, Hepatic panels, total protein, CK, uric acid, and LDH, temperature, pulse, blood pressure, respiratory rate, weight, vital signs

Twice weekly: (once discharged from hospital, optional) CBC with differential, Acute Care, Mineral, Hepatic panels, total protein, CK, uric acid, and LDH, reticulocyte count, prealbumin, complete vitals including weight, cyclosporine level

5 days after first dose alemtuzumab: serum troponin-I

After last dose of alemtuzumab: ECHO, 24 hour Holter, serum troponin-I, EKG

Weekly: EBV/CMV surveillance (starting day -28), C-reactive protein, stool for *C.difficile* toxin (if diarrhea occurs), drug levels where appropriate (e.g. gentamicin, vancomycin, cyclosporine).

Weeks -4, -1, day 0, weeks +2, +4, +6, +8, and +14: Peripheral blood for lymphocyte subset analysis. The sample for week -4 will be drawn before the first dose of alemtuzumab, and the day 0 sample will be drawn before stem cell infusion. A complete physical exam will be repeated weekly.

Days -28, -26, -24, -22, -19, -17, -15, -7, 0, +7, +14, +30, +45, +60, +100: Peripheral blood for pharmacokinetic studies to assess alemtuzumab metabolism. Blood draws on days of alemtuzumab administration will be performed 15 minutes before and 15 minutes after infusion of the drug

Days -1, +30, +60, and +100 (+/- 3 days): CT scans of the neck, chest, abdomen, and pelvis, photography of skin lesions, measurement of skin lesions when applicable and punch biopsies of skin lesions will be repeated to evaluate the status of the recipient's disease (only if skin lesions are accessible, evaluable or otherwise clinically indicated). All recipients will receive a repeat PFT on day +30 +/- 3 days.

Days +15, +30, +45, +60, and +100 (+/- 3 days): Peripheral blood will be drawn to assess for degrees of donor-host chimerism in the lymphoid and myeloid lines, to analyze lymphocyte subsets and to evaluate for the presence of a malignant T cell clone by PCR analysis of T-cell receptor gene rearrangement (where applicable but only on days +30 and +100 +/- 3 days). Peripheral blood will also be drawn at the time of GVHD and/or disease regression.

90 days (+/- 7 days) after the last dose of alemtuzumab, ECHO, serum troponin-I.

Day 100 +/- 3days: FDG-PET scan

6.3 Follow Up Beyond Day 100

Months 6, 12, 18, 24, 36, 48, and 60 (+/- 1 month): CBC with diff, Acute Care, Mineral, Hepatic panels, total protein, CK, uric acid, and LDH, peripheral blood for mixed chimerism studies and lymphocyte subset analysis, cortisol, TSH, T4, FSH (in females only), LH (in females only), testosterone/estradiol, pulmonary function testing and FDG-PET scans

Every two months x 3, then every 3 months for first 2 years, then every 6 months until year 5 post BMT(+/- 1 month): CT scans of neck, chest, abdomen, and pelvis, photographs of skin lesions, measurement of skin lesions when applicable and skin biopsies (if they have accessible or evaluable lesions or if otherwise indicated clinically). An apheresis of up to 10^{10} cells and skin biopsies of responding lesions may be performed at the time of clinical response (at the discretion of the PI). FDG PET every 6 months X 2 years and then annually until 5 years post transplant.

After 5 years follow up visits are not mandatory but yearly communication with the recipient and the referring physician is continued.

7 TREATMENT PLAN - RECIPIENT

7.1 Apheresis of recipient (optional)

One collection of 10^{10} cells by apheresis for cryopreservation of lymphocytes for later CTL microcytotoxicity assays and for baseline microsatellite PCR analysis for later use in chimerism studies. This procedure will be performed pretransplant (date not critical, but before the first dose of alemtuzumab) and at the time of disease response or acute graft vs. host disease (for laboratory investigation of graft-versus-malignancy, graft versus marrow, or GVHD effectors). Even if apheresis is not possible, research blood will be drawn.

7.2 Central Venous Catheter placement

A central venous catheter will be placed by interventional radiology or surgery pre-transplant (date not critical).

7.3 Infection Prophylaxis

Strongyloides prophylaxis: See Supportive Care Guidelines

Antibacterial Prophylaxis: See Supportive Care Guidelines

PCP Prophylaxis: To start at the time of alemtuzumab conditioning. Agents to be used and duration per Supportive Care Guidelines.

Antifungal Prophylaxis: See Supportive Care Guidelines

Candida Prophylaxis: Fluconazole 400 mg PO or IV once daily to be initiated at the start of conditioning (day -28). See Supportive Care Guidelines for aspergillus prophylaxis).

Antiviral Prophylaxis: See Supportive Care Guidelines

CMV reactivation: See Supportive Care Guidelines

Fever Regimen: See Supportive Care Guidelines

7.4 Preparative regimen (Appendix C)

Alemtuzumab 3mg IV on day-28
Alemtuzumab 10mg IV on day-27
Alemtuzumab 30mg IV on days -26, -24, -22, -19, -17, -15
Fludarabine 25mg/m²/d IV on days -5 to -1

7.5 GVHD prophylaxis

Recipients will start CSA 3 mg/kg/dose PO every 12 hours or 2 mg/kg/dose IV every 12 hours if unable to tolerate PO on day -4. Further management of immunosuppression will follow the guidelines outlined in Section 7.10.-

7.6 Transfusion support (see See supportive care guidelines)

Filtered and irradiated blood products will be used. CMV negative recipients of CMV negative or positive marrow to receive CMV negative blood products.

7.7 Nutrition

Parenteral nutrition will be instituted if clinically indicated.

7.8 Hospital Discharge

Recipients will receive the first three doses of alemtuzumab as inpatients or outpatients. All other doses of Campath and fludarabine can be administered on an outpatient basis unless severe reactions requiring close monitoring occur.

7.9 Donor Lymphocyte Infusions and Cyclosporine A

- 1) No DLI or withdrawal of immunosuppression if recipient has \geq grade II GVHD (See Supportive Care Guidelines for grading).
- 2) Failure to engraft- For the purposes of this protocol, sustained donor immune engraftment will be defined as the presence of detectable donor T cells on day 100. Those recipients with no detectable donor cells at any time \geq 30 days will be deemed to have graft rejection and will be taken off study.
- 3) Impending graft rejection- Declining donor T-cell chimerism below 50% despite at least 2 DLIs. Immunosuppression will be withdrawn in these recipients and disease status monitored without further manipulations to alter chimerism.
- 4) Any change in chimerism \leq 5% will be considered stable. Changes $>$ 5% will be considered increases or decreases as the case may be.
- 5) Recipients with stable mixed chimerism (50- 100% donor T-cell chimerism after 3 DLIs and withdrawal of immunosuppression) or complete donor chimerism AND stable or progressive disease after day 100 can receive immunomodulatory agents such as GM-CSF, IFN- α , IL-2 or additional DLIs at the discretion of the Principal Investigator (PI).

- 6) Recipients with Sezary syndrome will have T cell chimerism studies done on nonmalignant, circulating T-cells.

7.10 Guidelines for withdrawal of CSA and for administration of DLI:

Donor T-cell chimerism will be assessed on **day 30** +/- 7 days and will guide changes.

1) 100% donor T cell chimerism

CSA will be tapered from approximately day 60 in the absence of \geq grade II GVHD.

2) \leq 5% donor T cell chimerism

- a. No detectable ($< 1\%$) donor T or myeloid cells- Results will be confirmed and the subject taken off study.
- b. Detectable ($\geq 1\%$) donor T or myeloid cells- In the absence of \geq grade II GVHD, subjects will be maintained on CSA and receive a DLI. Chimerism will be rechecked in 1 month +/- 1 week.
 - Subjects with $> 5\%$ donor T cells at this time will receive dose escalated DLIs or have their immunosuppression altered as outlined below in sections 3 and 4.
 - Subjects who continue to have detectable donor T or myeloid cells with $\leq 5\%$ donor T cell chimerism will be eligible to receive another DLI. Chimerism studies will be repeated in 30 days (approximately day 100) to monitor the effects of the DLI. If after these maneuvers donor T cell chimerism continues to be $< 5\%$ additional DLIs with or without immunosuppression may be administered at the discretion of the PI.

3) $> 5\%$ and $< 50\%$ donor T cell chimerism

Recipients will be maintained on CSA and a DLI will be administered in the absence of \geq grade II GVHD. Chimerism will be rechecked in a month.

- a. If donor T cell chimerism continues to be $>5\%$ and $<50\%$, up to two more, dose escalated DLIs will be administered to try and convert recipients to complete donor chimerism in the absence of \geq grade II GVHD.
 - Those recipients whose donor T cell chimerism remains between 5% and 50%, can receive additional DLIs (either alone or preceded by immunosuppressive chemotherapy) at the discretion of the PI.
- b. If donor T cell chimerism becomes $\geq 50\%$ at any point, CSA will be tapered over two weeks, and dose escalated DLIs administered at intervals of approximately one month or more (up to a total of three) as required to achieve full donor T cell chimerism (in the absence of \geq grade II GVHD). If, after these interventions, donor T cell chimerism remains $\geq 50\%$ but $< 100\%$, further DLIs (beyond a total of 3- either alone or preceded by immunosuppressive chemotherapy) can be administered based on disease status at the discretion of the PI and GVHD

4) $\geq 50\%$ but $< 100\%$ donor T cell chimerism

In the absence of \geq grade II GVHD CSA will be withdrawn over a period of 2 weeks and chimerism repeated.

- a. If donor chimerism remains between 50% and 100%, up to three dose-escalated DLIs will be administered, usually at monthly intervals - in the absence of \geq grade II GVHD
- b. If, after these interventions, chimerism remains \geq 50% but $<$ 100%, further DLIs (beyond a total of 3-either alone or preceded by immunosuppressive chemotherapy) will be administered based on disease status at the discretion of the PI.

5) Threatened graft rejection – Those recipients whose donor T-cell chimerism falls below 50% after having been \geq 50%.

Recipients restart CSA or other appropriate immunosuppression and may- receive up to three doses of escalated DLIs at monthly intervals (in the absence of \geq grade II GVHD) in a bid to circumvent graft rejection. Additional DLIs with or without immunosuppression may be administered at the discretion of the PI.

8 DONOR EVALUATION AND PLAN

8.1 Pre-Study consult and evaluation

8.1.1 Pre-study related donor evaluation

- HLA, -A -B -DR typing to confirm HLA identity with the recipients
- History and physical examination
- Hepatitis B, Hepatitis C, HIV, HTLV-1, CMV antibodies, RPR, VZV serology, anti- T. cruzi and West Nile virus NAT (nucleic acid test)
- Chest X-ray in donors with underlying pulmonary disease or history of smoking
- CBC with differential, coagulation screen, acute care, hepatic, mineral screen panels and total protein, CK, uric acid, and LD
- Iso-heme titers
- Hemoglobin electrophoresis
- Type and antibody screen
- Toxoplasma PCR and EBV PCR
- If eligible: Orientation - visit to Department of Transfusion Medicine (DTM) - inspection of veins to determine need for central line versus peripheral IV for apheresis.
- Consent to undergo GCSF mobilization and donate leukocytes.
- Pregnancy test (in all females)
- Sickling hemoglobinopathies (HbSS, HbSC) by Hx or peripheral smear; HbAS acceptable
Cardiac function: EKG, 24 hour holter monitor, ECHO. All subjects age \geq 50, or age \geq 40, with one or more risk factors for coronary artery disease (history of high blood pressure, increased cholesterol, smoking, or diabetes or family history of coronary disease), will have a baseline cardiac consult and further testing per their recommendations.

8.1.2 Pre-study unrelated donor evaluation

- Unrelated donor matched at HLA-A, B, C, DRB1 and DQB1 loci by high resolution (10/10 allele match)
- Evaluation of donor suitability and eligibility will be performed by National Marrow Donor Program (NMDP) network or cooperative centers, in accordance with the most recent NMDP Standards (<http://bethematch.org/WorkArea/DownloadAsset.aspx?id=1960>) and according to the strictest interpretation of the latest FDA and AABB guidelines.

- Donor source documents are maintained at the NMDP Donor Center. Donor eligibility, clearance, consent, and any exceptions will be documented under the donor’s NMDP Donor ID number.
- Additional studies performed on blood sample at the time of donor collection include EBV IgG & IgM, EBV Early Antigen, Adenovirus Antibody, HSV Antibody, Toxoplasmosis Antibody, Hepatitis A Antibody, and Hepatitis A IgM. The results of these tests are for clinical post-transplant care of the patient, and do not impact donor selection.

8.2 First apheresis - Donor lymphocyte collection (pre-transplant)

To collect PBMC for DLI and for in vitro CTL activity assays.

8.3 Donor mobilization with G-CSF

8.3.1 Matched related Donor mobilization with G-CSF – per supportive care guidelines

After medical evaluation and clearance for suitability as an allogeneic donor by the BMT service in consultation with DTM, the donor will undergo mobilization with filgrastim (G-CSF) as an outpatient. Filgrastim (G-CSF) will be administered subcutaneously based on body weight (see below) starting day 4 before the apheresis. Dosing will be based on actual body weight unless the subject is “obese” (BMI > 35) when practical weight will be used. Filgrastim (G-CSF) will be administered according to a vial based algorithm to reduce wastage, improve donor compliance, and optimize CD34 yields. The doses for days 1-4 may be given at any time of day, but the doses for days 5 (and days 6 and 7 if needed) must be given very early in the morning, prior to apheresis. Predictable side effects of filgrastim (G-CSF), including headache, bone pain, and myalgia, will be treated with acetaminophen. Prophylactic treatment of these side effects with the same medications may be elected. Other side effects will be evaluated and treated accordingly.

Dose algorithms: Two dosing algorithms are recommended for use in the Clinical Center. The **standard dose algorithm** uses doses in the range of 10 to 12 mcg/kg /day, with a higher dose given to lighter weight adult donors to improve CD34+ yields. The **higher dose algorithm** is intended for use in (1) adult autologous donors who have received prior myelotoxic agents; (2) adult donors whose components will undergo further processing; (3) protocols in which a high transplant cell dose is required ($CD34 \geq 8 \times 10^6$); and (4) situations with large weight discrepancy between the adult donor and recipient (recipient > donor) (as assessed by the PI). The total dose in both regimens is capped at 1200 mcg/day. The PI or his designee will determine the dosing algorithm to be utilized.

I. Standard-Dose Filgrastim Algorithm

Donor Weight	Total Daily Filgrastim	Dose (range)
38 - 60 kg	600 mcg	(10.0 to 15.8 mcg/kg)
61 - 78 kg	780 mcg	(10.0 to 12.8 mcg/kg)
79 - 90 kg	900 mcg	(10.0 to 11.4 mcg/kg)
91 - 96 kg	960 mcg	(10.0 to 10.5 mcg/kg)
97 - 108 kg	1080 mcg	(10.0 to 11.0 mcg/kg)
≥ 109 kg	1200 mcg	(11.0 or less)

II. Higher-Dose Filgrastim Algorithm

Donor Weight	Total Daily Filgrastim	Dose (range)
38 - 48 kg	600 mcg	(12.5 to 15.8 mcg/kg)
49 - 56 kg	780 mcg	(13.9 to 15.9 mcg/kg)
57 - 60 kg	900 mcg	(15.0 to 15.8 mcg/kg)
61 - 67 kg	960 mcg	(14.3 to 15.7 mcg/kg)
68 - 108 kg	1080 mcg	(10.0 to 15.9 mcg/kg)
≥109 kg	1200 mcg	(11.0 or less)

8.3.2 Adult donor mobilization with G-CSF (> 18 years old) / matched unrelated donor – per NMDP Guidelines

Unrelated donors will be mobilized using G-CSF per NMDP guidelines. Apheresis will be completed per NMDP standards and in accordance with most recent and stringent FDA guidelines.

8.4 Peripheral Blood Stem Cell Collection & Processing

8.4.1 Peripheral Blood Stem Cell Collection & Processing for matched related donor

Donors will receive divalent cation prophylaxis to prevent citrate toxicity during apheresis, in accordance with standard DTM policies. The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, based on peak CD34+ cell mobilization response to G-CSF and the CD34+ cell dose needed, based on kilogram weight of recipient. In pediatric subjects, defined as less than 40 kg, a maximum of 8 blood volumes will be processed per day, for up to 2-3 days. Filgrastim may be continued and apheresis may be repeated on days 2 and 3 if needed to meet the target dose of CD34+ cell dose.

The volume of the leukapheresis procedure will be targeted to obtain a progenitor cell dose of 5×10^6 CD34+ cells/kg. Obtaining a CD34+ dose of $> 3 \times 10^6$ cells/kg will be considered adequate and no further apheresis will be performed. If after the first day of apheresis, the product contains $< 3 \times 10^6$ /kg CD34+ cells, a decision regarding further apheresis will be made at the discretion of the PI, in consultation with DTM. The minimum acceptable CD34+ cell dose to proceed with the protocol is 2×10^6 /kg.

If the minimum CD34+ cell dose is not achieved with one mobilization and 3 apheresis collections (i.e. 2×10^6 CD 34/kg), then the PI will consider options of a bone marrow collection, a second course of mobilization and apheresis, or no further donor collections and taking the subject off protocol.

The PBSC collections will not be manipulated except for plasma removal or red blood cell depletion as needed for ABO or other red blood cell antigen incompatibility. PBSC will be either cryopreserved for later thawing and infusion, or infused as fresh products within 48 hours of collection.

On rare occasions, subjects (recipients) may develop marrow failure as a consequence of graft vs. host hematopoiesis (GVH) or from post transplant drug treatment (i.e. ganciclovir). When such situations arise, subjects may require a stem cell “boost”. Donors will undergo a repeat mobilization

with G-CSF (as outlined above) followed by CD34+ selection of hematopoietic progenitor cells prior to being infused.

8.4.2 Peripheral Blood Stem Cell Collection & Processing for matched unrelated donors

Unrelated donors will be collected per NMDP policies and procedures. Donors will not be collected prior to patient screening and enrollment on the protocol.

The unrelated donor will undergo G-CSF mobilization and Peripheral Blood Stem Cell (PBSC) collection. The volume of the leukapheresis procedure will be targeted to obtain a progenitor cell dose of 5×10^6 CD34+ cells/kg. Obtaining a CD34+ dose of $> 3 \times 10^6$ cells/kg will be considered adequate. The minimum acceptable CD34+ cell dose at the NMDP center to proceed with the protocol is 2×10^6 /kg.

The product will be collected at the NMDP Donor Center and transported by courier to NIH per NMDP guidelines. The product will be processed at NIH and cryopreserved (see 8.4.1).

9 MANAGEMENT OF RECIPIENT COMPLICATIONS

The major complications are CMV reactivation, acute and chronic GVHD, disease progression, graft failure, and opportunistic infections (bacterial/fungal). Subjects with these complications will be treated according to the BMT consortium Supportive Care Guidelines or as outlined below:

9.1 CMV reactivation (See Supportive Care Guidelines)

9.2 Acute GVHD (grade 3 or 4) (See Supportive Care Guidelines)

9.3 Chronic GVHD (see Supportive Care Guidelines)

- Continue or restart cyclosporine at standard dose
- Prednisone, dose according to severity
- Penicillin V 500mg PO BID for bacterial prophylaxis
- Bactrim three times a week while the subject is on prednisone therapy for chronic GVHD
- Change to alternate day steroid and CSA therapy when response is established.
- Non-responding subjects may be treated with other standard of care therapies such as azathioprine, sirolimus, tacrolimus, mycophenolate, basiliximab, lenalidomide infliximab, PUVA, thalidomide and/or other agents at the discretion of the attending physician. Antifungal prophylaxis with voriconazole with prednisone will be started at the discretion of the PI depending on the dosage and duration of therapy with steroids

9.4 Disease Progression

Subjects demonstrating disease progression may be treated with donor lymphocyte transfusions as outlined in section 7.9.

Subjects with progressive disease may be treated with interferon-alpha, IL-2, GM-CSF and or chemotherapy at the discretion of the attending physician.

9.5 Graft Rejection

This transplant protocol uses a nonmyeloablative preparative regimen. Therefore, auto-recovery is anticipated in recipients who fail to engraft. Recipients who fail to demonstrate donor engraftment despite all reasonable attempts (Section 7.9 and 7.10) will be taken off study and referred back to their primary physicians for further therapy.

9.6 Graft Failure

If the marrow becomes aplastic the recipient will receive another G-CSF mobilized, CD34 selected PBPC transplant from the same donor. The recipient will receive G-CSF after this PBPC infusion to expedite hematologic recovery.

9.7 Donor-recipient ABO incompatibility (See Supportive Care Guidelines)

Minor ABO incompatibility can cause profound hemolysis of recipient red cells in the first two weeks after transplant because of the rapid production of donor anti-A and anti-B antibodies.

9.8 Pulmonary Engraftment Syndrome

Recipients who develop pulmonary engraftment syndrome (around 10-14 days post-transplant) will be treated with steroids.

10 STUDY MODIFICATIONS

10.1 Use of donor lymphocytes following development of acute GVHD

Recipients developing grade II or greater GVHD after transplant or after lymphocyte infusion will not receive further lymphocyte infusions unless GVHD has been successfully treated (i.e. off steroids) and tumor progression is documented.

11 RESPONSE CRITERIA

Complete response (CR) - disappearance of all signs and symptoms of CTCL for a period of at least one month.

Partial response (PR) - a 50% or greater decrease in the sum of the products of the longest perpendicular diameters of all measured lesions (a greater than 50% reduction in area of disease involvement in the case of cutaneous disease) lasting for a period of at least one month. No new metastatic lesions may appear.

Stable disease (SD) - tumor measurements not meeting the criteria of CR, PR, or PD

Progressive disease (PD) - increase of 25% or greater in the sum of the products of the longest perpendicular diameters of all measured lesions (a greater than 25% increase in area of disease

involvement in the case of cutaneous disease) compared to the smallest previous measurements, or the development of any new metastatic or cutaneous disease.

12 ANCILLARY LABORATORY RESEARCH STUDIES

12.1 Intended use: During the course of participating on this study, blood and tissue will be collected for correlative laboratory research studies. These specimens will not be read by a pathologist or used for diagnostic purposes. Studies will not be used in assessing the primary endpoint but are undertaken for descriptive or exploratory ancillary research, which are approved by the NHLBI IRB and listed in the Appendix E of the protocol.

- **GVT and GVH studies:** Blood and serum from recipients and donors will be obtained by leukapheresis to study genomic and proteomic pathways involved in mediating GVHD and in vitro donor T-cell responses to the recipient's tumor as well as normal cells. Biopsies of accessible lesions and/or apheresis of recipients with circulating Sezary cells will be performed to aid in GVT studies. Regressing tumors will be biopsied when accessible, to investigate the presence of donor derived TIL.
- **Engraftment :** PCR-based microsatellite analysis will be performed at various time points after the transplant to assess the degree of donor lymphoid and myeloid chimerism
- **Immune Reconstitution:** Lymphocyte subset analysis and T cell repertoire studies will be performed at various time points after the transplant to assess immune reconstitution
- **Pharmacokinetic studies on alemtuzumab:** Circulating alemtuzumab levels may be measured at multiple time points after alemtuzumab administration to assess the half-life of the drug. The alemtuzumab 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. 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).
- **Chimerism studies:** PCR of microsatellites of post-transplant peripheral blood T-lymphocytes, B-cells, NK cells and blood myeloid cells will be assessed in serial fashion to determine the chimeric status of the patient as a function of time. These studies will be repeated after lymphocyte infusions to assess their effect on host chimeric status as well. The relationship between degrees of donor host chimerism, GVHD and tumor response will be analyzed.
- **Pulmonary function tests:** Pulmonary status at baseline, day 30 and after day 100 will be analyzed to see if reduced intensity conditioning (RIC) effects lung function before day 100 or if the decline in lung function is only seen later consistent with bronchiolitis obliterans syndrome (BOS).
- **Other research labs as described on the** List of Laboratory Studies Using Human Subject and Normal Volunteer Samples in the Hematology Branch submitted to and reviewed by the IRB (See Appendix E)

- 12.2 Storage:** Research samples will be stored with identifiers in the secure laboratory of Dr. Richard Childs, Senior Investigator, Hematology Branch, NHLBI.
- 12.3 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.
- 12.4 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.
- 12.5 Loss or destruction of samples:** Should we become aware that a major breach in our plan for tracking and storage of samples has occurred, the IRB will be notified.
- 12.6 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. 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).

13 BIOSTATISTICAL CONSIDERATIONS

13.1 Sample Size Determination

The study will accrue up to 25 recipients and 25 donors (see sections 13.3).

13.2 Parameters to be monitored

- 1) Tumor size (Clinical evaluation of skin lesions, photography and CT scans on days -1, +30, +60, and +100)
- 2) Disease response (biopsy of accessible and evaluable lesions or if clinically indicated on days -1, +30, +60, +100, and 5, 7, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, and 60 months and/or at the time of observed regression)
- 3) CD34+ dose
- 4) CD3+ dose
- 5) Lymphocyte subset analysis (CD3+, CD4+, CD8+, CD45RA, CD45RO+, HLA DR, CD57, CD25, NK cells, B cells) and lymphocyte recovery levels
- 6) Genotypic evaluation of dermal and circulating neoplastic T cells (PCR analysis of TCR gamma chain)
- 7) Degrees of donor-recipient lymphoid, NK cell and myeloid chimerism as a variable of time and after lymphocyte infusions by PCR analysis of microsatellites in peripheral blood lymphocytes and myeloid cells
- 8) Neutrophil recovery (days to neutrophil count of 0.5×10^9 and $1.0 \times 10^9/l$).
- 9) Platelet recovery (days to platelet count of $50 \times 10^9/l$, days to transfusion independence).
- 10) Red cell recovery (days to transfusion independence).
- 11) Evidence for a GVT effect after cyclosporine taper and lymphocyte infusions.

- 12) Effects of lymphocyte infusions on donor-host chimerism measured by microsatellite DNA analysis of lymphocytes and myeloid cells.
- 13) Incidence and severity of acute GVHD.
- 14) Incidence and severity of chronic GVHD.
- 15) Non-hematologic effects attributable to the preparative regimen.
- 16) Disease-free survival and overall survival.
- 17) Alemtuzumab half-life if required and done
- 18) Transplant related mortality (day +100 and day +200).
- 19) CMV reactivation
- 20) Cause of death.

13.3 Goals

This trial was originally designed as a Phase I-II study. The primary goal was to find the minimal dose of conditioning regimen (of the two considered) which would have resulted in engraftment in greater than 80% of patients. If such a dose were found, our goal was to assess efficacy and our plan was to accrue enough subjects to estimate the proportion of response (CR or PR) to within $\pm 20\%$ and to rule out a proportion of response of 10% or less if no subjects responded.

In May 2009, the sample size of the study was decreased and the study was redesigned to assess the confidence interval for efficacy as detailed below. The sample size was reduced from 58 to 25 recipients and the plans for multiple testing procedure for engraftment and dose escalation were eliminated.

Confidence interval for efficacy

Given the slow accrual in this rare disease, we wish to assess efficacy and we will accrue enough subjects to estimate the proportion of complete response (CR) with a two-sided 95% confidence interval of a width less than 40%. We will use the two-stage design of Gehan [90] to estimate the proportion of CRs. Initially we will accrue 14 subjects and then add up to 11 more subjects, depending on the number of CRs achieved.

The numbers of subjects needed in the second stage of the study are summarized in the following table:

Number of CRs observed among 14 subjects in Stage 1	Number of subjects needed in Stage 2	Total number of subjects in the study
0-1 CR	+ 0 subject	14 subjects
2 CRs	+ 1 subject	15 subjects
3 CRs	+ 5 subjects	19 subjects
4 CRs	+ 8 subjects	22 subjects
5-9 CRs	+ 11 subjects	25 subjects
10 CRs	+ 8 subjects	22 subjects
11 CRs	+ 5 subjects	19 subjects
12 CRs	+ 1 subject	15 subjects
13-14 CRs	+ 0 subject	14 subjects

The above design will allow us to estimate a 95% confidence interval for the proportion of CRs with a width <40%. We plan to include the first two subjects accrued to the earlier version of this protocol in the total sample size.

Statistical methods:

The planned analyses will include descriptive statistics on the proportions of response and 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 exact method based on Binomial distributions or normal approximation, if appropriate. The transplant outcome by donor type (related vs. unrelated) will be compared. Analyses based on survival analysis and regression methods will also be employed if deemed appropriate.

13.4 Bayesian Stopping Rule for Day 100 Transplant-Related Mortality (TRM)

To monitor TRM, we adopt a Bayesian approach that formally incorporates our “prior” expectations about 100-day TRM (Geller et al. 2004 [98]). From prior experience we anticipate the 100-day NRM rate should not be higher than 20%. A stopping boundary is reached if the true rate exceeds the anticipated rate with a probability at least 0.90. We take our prior distribution to be a beta distribution with a mean of 0.20 and the sum of the two beta parameters to be 6, meaning that we take our prior beliefs to be “worth” 6 patients. This implies that the parameters of the beta prior distribution are 1.2 and 4.8 (mean 0.20 and variance 0.0229). The stopping boundaries are given in the table below.

Number of transplant recipients	Stop if # of NRM by day 100 reaches or exceeds
Up to 5	3
6-8	4
9-12	5
13-16	6
17-20	7
21- 25	8

This stopping rule was assessed by simulation. Based on 100,000 repetitions, the probability of meeting the stopping boundary was .19 under the null hypothesis of $p_{TRM} = 0.20$ and 0.88 under the alternative hypothesis that $p_{TRM} = 0.40$. We concluded that the likelihood of stopping was satisfactory to protect patient safety for 100 day TRM.

13.5 Drop outs

Once recipients undergo the stem cell transplantation they will be evaluated for engraftment and 100 day TRM. Anyone treated on this protocol will be counted.

14 REMOVAL FROM THE STUDY

14.1 Withdrawal from the transplant procedure

Recipients and their donors will be given ample time to withdraw from the study prior to admission for transplant. Thereafter, the nature of the procedure does not permit safe withdrawal from the protocol.

14.2 Withdrawal from Experimental Protocol

The recipient and donor have the right at any time to elect not to participate in the research aspects of the protocol.

Recipients who fail to achieve allogeneic marrow engraftment (donor chimerism or mixed chimerism) will be removed from study after full autologous marrow recovery is achieved.

Recipients with disease progression associated with a significant decline in performance status which negates further treatment on protocol (i.e. donor lymphocyte infusion) will be removed from study.

15 DATA SAFETY AND MONITORING PLAN

15.1 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 subject consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to the 45 Code of Federal Regulations (CFR) 46 (Protection of Human Subjects). This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

DSMB: The NHLBI Data safety and Monitoring Board will review the protocol at six or twelve month intervals. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

15.2 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.

15.2.1 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: unexpected in terms of nature, severity, or frequency in relation to the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and the characteristics of the subject population being studied; and related or possibly related to participation in the research; and places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

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:

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

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.

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

Event Characterization and Reporting to the IRB and Clinical Director (CD)

Approved by HSRAC on September 30, 2013

Date effective: October 28, 2013

All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, and serious protocol deviations, will be reported to the IRB and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and Clinical Director as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event. SAEs that do not meet the criteria of Unanticipated Problem (UP) must be reported to the IRB Chair and Clinical Director through the NHLBI Protocol Tracking Management System (PTMS) within 14 days of learning of the event using the SAE form in PTMS.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

15.3 Adverse Events for transplant recipients

Adverse events used to evaluate the safety of this protocol regimen will be collected to include any unfavorable and unintended signs (including abnormal laboratory findings), symptoms or diseases (i.e. incidence of GVHD, graft failure, regimen related toxicities, or infectious complications) which either occur during the study having been absent at baseline or if present at baseline appear to worsen with **the following exceptions**. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 3.0. A copy of the criteria can be down-loaded from the CTEP home page:

<http://ctep.cancer.gov/reporting/ctc.html>

The following expected outcomes will be documented in the subject's medical record. A table of non serious adverse events will be provided to the IRB at the time of continuing review. Events will not be reported to the IRB via the expedited method unless they meet the criteria of a severe adverse event (SAE):

- Renal insufficiency
- Hepatic insufficiency
- Transient cardiac arrhythmias
- Transient cardiac insufficiency
- Pulmonary insufficiency
- Neutropenia and its complications
- Thrombocytopenia and its complications
- Anemia and its complications
- Transfusion reactions
- Treatable infections from bacteria, viruses, protozoa and fungi
- Late effects of transplant regimens including: cataracts, infertility, growth impairment, hypothyroidism, and dental caries
- Headache, insomnia, psychosis, mood changes, disorientation, seizures from metabolic imbalance
- Nausea, vomiting, diarrhea, mucositis, weight loss, dry mouth, hiccoughs, constipation
- Well-characterized drug reactions - allergic manifestations, "red man" syndrome

- Well-characterized drug side effects from drugs used routinely in transplant recipients (e.g.; preparative regimen chemotherapy, immunosuppressive drugs, antimicrobials)
- Common side effects of antiemetics, analgesics, anti-inflammatory agent and known complications of steroid therapy
- Complications from intravenous catheters, thrombotic occlusion, infection, local reactions, cardiac arrhythmia

The following expected outcomes will not be reported to IRB at each occurrence unless they meet the criteria of an SAE. The PI will incorporate these events into the protocol and consent as appropriate. They will be reported in summary form at the time of continuing review and at termination of the protocol.

- Acute graft-versus-host disease
- Chronic graft-versus-host disease
- Graft failure / graft rejection
- Veno-occlusive disease
- Hemorrhagic cystitis
- Regimen-related toxicity
- Cytomegalovirus reactivation and disease
- Disease relapse or progression
- Secondary hematologic and/or solid tumor malignancy
- Pre-malignant conditions such as myelodysplastic syndrome

In addition to the adverse event reporting exclusion listed above, grade 1 events will not be reported to the IRB at the time of continuing review.

15.4 Adverse Events for Donors

The following are expected outcomes of for the donor that will be reported to the IRB in tabular form at the time of continuing review unless they fulfill the criteria for a SAE:

- bruising at the site of venipuncture
- common side effects of G-CSF administration (bone pain, fatigue, arthralgias, headache, insomnia, fever, worsening of pre-existing skin rashes, increases of alkaline phosphatase, lactate dehydrogenase and/or uric acid levels, elevated blood leukocyte count, thrombocytopenia)
- Hypotension during apheresis

The following expected outcomes will not be reported to the IRB at each occurrence unless they meet the criteria of a SAE. The PI will incorporate these events into the protocol and consent as appropriate and they will be reported in summary form at the time of continuing review and at termination of the protocol:

- Ischemic chest pain during G-CSF administration
- Splenic enlargement
- Bone pain, muscle aches or headaches not controlled with non-narcotic analgesics
- Cutaneous vasculitis

The following serious adverse events will be listed in the consent form and reported to the IRB as outlined in section 15.5

- Ischemic chest pain during G-CSF administration that requires transfer to the intensive care unit
- Splenic rupture
- Anaphylaxis or allergic reactions requiring intravenous or subcutaneous epinephrine

In addition to the adverse events reporting exclusion listed above, grade 1 events will not be reported to the IRB at the time of continuing review.

15.5 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/research nurses and/or a contracted data manager will assist with the data management efforts.

All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded in DIR's Clinical Data System (CDS) or the Laboratory of Cardiac Energetics (LCE) database. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., study-specific identifying number (SSPIN) generated by CDS or other unique code or minimum PII required for subject identification.

CIBMTR: For the purposes of quality assurance (i.e. accreditation of the Transplant program), anonymized data will be released to the Center for International Blood and Marrow Transplant Research (CIBMTR) according to federally mandated policies and procedures.

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 breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

Publication Policy: Given the research mandate of the NIH, subject 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 (OHSR).

16 HUMAN SUBJECT PROTECTIONS

16.1 Rationale for Subject Selection

No subjects will be excluded from participation based on gender, race or ethnicity. All patients with mycosis fungoides (MF) or Sezary syndrome (SS) staged as outlined in section 5.1 will be considered for the protocol. Only patients that are considered unfit to withstand a nonmyeloablative transplant as outlined in section 5.3, will be excluded.

Strategies for subject recruitment: Hematologists and oncologists throughout the country will be informed about our protocol by letter.. Information about the protocol will be posted on the NHLBI patient recruitment website, NCI website (cancer.gov), Cutaneous Lymphoma Foundation website and on ClinicalTrials.gov. We are also in the process of initiating collaboration with the CTCL Clinic, Washington Hospital Center, to serve as a source of patient referrals. Additionally, we will also advertise in relevant medical journals.

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the subjects are diagnosed with a rare disease; the subject population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

Payment for participation: \$0

16.2 Participation of children

In principle, age is not a consideration, but in practice we are limiting the protocol to subjects 18-70 years of age (both inclusive) as MF/SS are diseases largely afflicting adults, with most patients being 40-60 years at diagnosis.

16.3 Risks and Discomforts

16.3.1 The recipient

Related to the transplant procedure: Bone marrow stem cell transplantation is a major procedure, which entails serious discomforts and hazards for the recipient. Although we anticipate that this protocol is relatively safe, the mortality from conventional BMT may be as high as 40%. It is therefore only appropriate to carry out this experimental procedure in the context of a life threatening condition and with full informed consent from the recipient, and donor. The major hazards (GVHD, infection, graft failure, and disease progression) have already been discussed. The major discomforts are those of nausea, mucositis, anorexia, diarrhea, fever and malaise. The specific hazards of this study using a nonmyeloablative preparative regimen in the context of a PBPC transplant are: graft rejection, GVHD, and disease progression.

Transplant recipients have been reported to have an increased risk of solid cancers but most studies are small and have limited ability to evaluate the interaction of host, disease, and treatment-related factors. In the largest study to date to evaluate risk factors for solid cancers, we studied a multi-institutional cohort of 28874 allogeneic transplant recipients with 189 solid malignancies. Overall, patients developed new solid cancers at twice the rate expected based on general population rates (observed-to-expected ratio 2.1; 95% confidence interval 1.8-2.5), with the risk increasing over time (P trend < 0.001); the risk reached 3-fold among patients followed for 15 years or more after transplantation. New findings showed that the risk of developing a non-squamous cell carcinoma (non-SCC) following conditioning radiation was highly dependent on age at exposure. Among patients irradiated at ages under 30 years, the relative risk of non-SCC was 9 times that of non-irradiated patients, while the comparable risk for older patients was 1.1 (P interaction <.01). Chronic graft-versus-host disease and male sex were the main determinants for risk of SCC. These data indicate that allogeneic transplant survivors, particularly those irradiated at young ages, face increased risks of solid cancers, supporting strategies to promote lifelong surveillance among these patients. (Blood. 2009; 113:1175-1183).

Secondary MDS/AML of donor origin is also a known but rare complication following allo-transplant [101-104]. This phenomenon is increasingly being realized given our ability to do chimerism assays to distinguish donor from patient cells.

Side effects of common drugs used in this transplant regimen include:

Alemtuzumab: Adverse events noted on trials utilizing alemtuzumab and occurring in >5% of patients include nausea, vomiting, fever, chills, rigors, hypotension, rash, headaches, abdominal pain, myalgias, arthralgia, diarrhea, dyspnea, bronchospasm, angioedema, tumor lysis syndrome, infections, loss of appetite, hypertension, heart rhythm abnormalities including supraventricular tachycardia, dizziness, dysesthasias, tremors, stomatitis, insomnia, depression, cough, impaired sperm motility and/or EBV related lymphoproliferative disease.

The following is the black box warning for Campath-1 H

WARNING: CYTOPENIAS, INFUSION REACTIONS, and INFECTIONS

Cytopenias: Serious, including fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia can occur in patients receiving Campath. Single doses of Campath greater than 30 mg or cumulative doses greater than 90 mg per week increase the incidence of pancytopenia.

Infusion Reactions: Campath administration can result in serious, including fatal, infusion reactions. Carefully monitor patients during infusions and withhold Campath for Grade 3 or 4 infusion reactions. Gradually escalate Campath to the recommended dose at the initiation of therapy and after interruption of therapy for 7 or more days.

Infections: Serious, including fatal, bacterial, viral, fungal, and protozoan infections can occur in patients receiving Campath. Administer prophylaxis against *Pneumocystis jiroveci pneumonia* (PCP) and herpes virus infections

Infusion reactions: Alemtuzumab can result in serious, and in some instances fatal, infusion reactions. Patients should be carefully monitored during infusions and alemtuzumab discontinued if indicated.

Cardiac: Potential cardiac toxicity might be irreversible. For this reason, we will monitor subjects with an echocardiogram, 24 hour holter, and serum troponin level before treatment begins and after the last dose of Campath. The ECHO and the serum troponin will be repeated 90 days (+/- 7 days) after the last dose of alemtuzumab.

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

Infections, opportunistic infections: Serious, sometimes fatal bacterial, viral, fungal, and protozoan infections have been reported in patients receiving alemtuzumab therapy.

Numerous other adverse events have occurred in patients receiving therapy with single agent alemtuzumab, albeit at lower frequencies and with uncertain attributability to the agent. Of note, published case reports describe transformation of low-grade/indolent lymphomas to more aggressive variants, and occurrence of disorders related to immune dysregulation such as sarcoidosis.

Cyclosporine A: CSA is metabolized primarily in liver but the major toxicity is renal. Side effects include renal impairment, reversible renal insufficiency, hemolytic uremic syndrome, elevated bilirubin and transaminases that normalize with continued administration or reduced dose, hypertrichosis, headaches, nausea, gingival hypertrophy, parasthesias (painful hands and feet), hypertension, hypomagnesium, bilirubinemia, tremor, and seizure. An extremely rare complication of cyclosporine is blindness, which may be irreversible. Posterior Reversible Encephalopathy Syndrome (PRES) is an increasingly recognized neurologic disorder seen in 1% of patients on cyclosporine which manifest with acute to subacute hypertension and/or seizures [91]. In the event of hypertension, subjects will be prescribed 1 or more medications to control blood pressure in an effort to decrease the risk of this complication.

Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, and rarely hemolysis and pulmonary toxicity, hemolytic anemia and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.

The following is the black box warning for fludarabine

WARNING: CNS TOXICITY, HEMOLYTIC ANEMIA, AND PULMONARY TOXICITY
Severe central nervous system toxicity occurred in 36% of patients treated with doses approximately four times greater (96 mg/m²/day for 5 to 7 days) than the recommended dose. This toxicity was seen in ≤0.2% of patients treated at the recommended dose levels (25 mg/ m²). Instances of life-threatening and sometimes fatal autoimmune hemolytic anemia have been reported after one or more cycles of treatment. In a clinical investigation of the combination of fludarabine phosphate with pentostatin (deoxycoformycin) for the treatment of refractory chronic lymphocytic leukemia (CLL), there was an unacceptably high incidence of fatal pulmonary toxicity.

Risks related to pregnancy and breastfeeding: Pregnant and nursing women are excluded from accrual. Subjects are required to use effective contraceptive measures to prevent pregnancy during protocol participation to eliminate the possibility of drug effects on a developing fetus. Following transplantation, woman of childbearing age will be counseled regarding when it is safe to consider becoming pregnant should they so choose. Female subjects will be advised that should pregnancy occur, the research team must be informed immediately. An SAE will be filed on any pregnancy and this report will be followed up with information on the outcome of the pregnancy and complications of (should there be any).

16.3.2 The donor

Related to filgrastim (G-CSF): The hazard to the donor is low. The discomfort from G-CSF mobilization and leukapheresis for collection of PBPC are probably lower than those associated with marrow harvesting. G-CSF has been given to large numbers of donors without major side effects or long-term consequences. The immediate side effects of G-CSF are bone pain, fatigue, insomnia, myalgia and headache which are usually mild and self-limiting. Reversible thrombocytopenia, with platelet counts falling to the range of 100,000 / mm³ is common. Two patients have been reported to experience non-fatal splenic rupture after more prolonged treatment with higher doses of G-CSF. One of these two patients had concurrent mononucleosis, a second cause for splenic rupture. Donors will be asked to avoid vigorous activities and to report any left upper abdominal and/or shoulder pain to the research team or the on-call physician for the NIH Department of Transfusion Medicine at 301-496-1211. Other side effects include fever (rare, less than 1% of patients), worsening of pre-existing skin rashes and transient and reversible elevation of liver enzymes in the blood (suggesting transient

inflammation of the liver).

Patients with ongoing ischemic heart disease have been reported to have angina seemingly temporally related to G-CSF administration and apheresis. (In addition, a rare occurrence of pulmonary hemorrhage has been reported in a healthy donor who was a cigarette smoker and had underlying pulmonary disease [92].

Related to central line placement: It is estimated that approximately 50% of donors will require central venous line placement to successfully complete apheresis. Intravenous line placement in a large - vein using a temporary - catheter carries a small risk of bleeding, bruising or pain and a very low risk of accidental injury to the adjacent artery or nerve. These risks are minimized by using only trained, experienced MICU or interventional radiology staff for this procedure.

Related to leukapheresis: Adverse reactions related to leukapheresis include hypotension resulting from transient blood volume loss and cutaneous paresthesia from the use of anticoagulant. The former toxicity can be corrected by postural changes and volume replacement. The latter is manageable with slowing the rate of anticoagulant and/or providing calcium supplement. In exceptional circumstances the donor will be required to donate PBPC a third time or to give bone marrow. There is a minimal additional risk that the donation of PBPC on three successive days can increase the possibility of thrombocytopenia. This thrombocytopenia is transient and unlikely to cause clinical sequelae. There is no additional risk to giving bone marrow after PBPC donation (than would normally be associated with bone marrow harvesting).

Related to skin biopsies: Punch biopsies will be performed by the principal investigator or by a credentialed associate investigator or member of the Dept. of Dermatology or Surgery. Risks include pain, bleeding, hematoma, and infection at the site of biopsy.

16.4 Risks in Relation to Benefit

For Transplant Patients

Allogeneic PBSCT is curative in many patients with a variety of hematologic malignancies. However, conventional dose intensive transplantation is fraught with risks, including a mortality rate that can approach 50%. By utilizing a reduced intensity conditioning regimen, we aim to decrease morbidity and mortality associated with conditioning, thereby potentially improving outcome.

The approach outlined in this protocol is ethically acceptable because we will target a group of patients with an incurable malignancy who have made an informed decision to opt for a procedure which they understand may offer a chance of disease remission but with a risk of death from treatment failure or treatment-related causes. Implicit in this decision is the opportunity to weigh all treatment options after full information has been provided to them by the NIH team and the referring physician. Stopping rules will minimize the risk of untoward or unexpected side effects.

Therefore, in this study, the research involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects (45CFR 46).

As of February 21, 2017, this study is now closed to new subject accrual and continues in subject follow up only. Follow up under this protocol involves procedures that are more than minimal risk; therefore, the risk remains unchanged.

For Adult Donors:

Healthy HLA matched family members will be co-enrolled into this study as stem cell donors. The stem cell collection aspect of this protocol is not investigational. Despite the risks associated with this procedure (section 16.3.2), potential benefit does exist for family donors. The donor derives psychosocial benefit from donating stem cells both at the time of donation and possibly into the future, especially in view of the reduced life expectancy due to this disease in a family member. Other potential benefits include detection of illnesses, determination of blood cell counts, and evaluation of kidney and liver function in the potential donor at the time of screening.

Therefore, in this study, the research involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects (45CFR 46).

16.5 Consent Processes and Procedures

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study. Dr. Aue will lead this discussion.

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

We anticipate the enrollment of non-English speaking research participants into our study. The IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2. 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 3 participants in a given language and 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 3, 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.

16.7 Conflict of Interest

The Principal Investigator ensured 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.

17 PHARMACEUTICALS

17.1 ALEMTUZUMAB

Generic: alemtuzumab

02-H-0250
Amendment V
Georg Aue, M.D.
December 28, 2017

Classification: monoclonal antibody

Action: Monoclonal antibody directed against CD52 antigen, a surface glycoprotein expressed by lymphocytes

Availability: Commercial: Berlex Laboratories

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.

Stability: 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. 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.

Dose: 30 mg IV three times a week after initial doses of 3mg and 10mg

Side effects: See section 16.3, Risks and discomforts

17.2 CYCLOSPORINE (Gengraf, Sandimmune, Neoral)

Supply/product description – Cyclosporine will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (25 mg and 100 mg), USP [MODIFIED], oral solution (100 mg/ml), USP [MODIFIED], and as a parenteral concentrate for injection (50 mg/ml). When oral capsules are prescribed for this protocol, the cyclosporine capsules, USP [NON-MODIFIED] should NOT be used.

Preparation – For parenteral doses, each milliliter of concentrate (50 mg/ml) should be diluted in 20 to 100 ml of dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of cyclosporine will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing. Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.

Storage and Stability – Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light. Cyclosporine concentrate for injection that has been diluted to a final concentration of approximately 2 mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.

Administration – Cyclosporine may be given intravenously or orally.

Toxicities: Refer to section 16.3 Risks and Discomforts.

17.3 FLUDARABINE PHOSPHATE (Fludara)

Supply: commercially available

Product description: Fludarabine phosphate is commercially available as both a lyophilized powder for injection in vials containing 50 mg of fludarabine phosphate with mannitol 50 mg and sodium hydroxide for pH adjustment and a solution for injection in 2 ml vials containing 50 mg of fludarabine phosphate (25 mg/ml of fludarabine) with 25 mg/ml mannitol and sodium hydroxide for pH adjustment.

Preparation: Fludarabine lyophilized powder for injection should be reconstituted with 2 ml of sterile water for injection, USP to a concentration of 25 mg/ml. The prescribed dose of fludarabine should be diluted in 100 ml of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration over 30 minutes.

Storage and Stability: Fludarabine vials should be stored under refrigeration between 2° to 8° C (36° to 46° F). Reconstituted fludarabine phosphate is chemically and physically stable for 24 hours at room temperature or for 48 hours if refrigerated. The manufacturer recommends use of either the reconstituted powder for injection or the solution for injection (once diluted for administration) within 8 hours because neither product contains an antimicrobial preservative.

Administration: The prescribed dose of fludarabine should be diluted in 100 ml of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration over 30 minutes.

Toxicities: Refer to section 16.3 Risks and Discomforts.

17.4 FILGRASTIM (G-CSF, Neupogen)

Supply: Commercially available.

Product description: Filgrastim injection is available in a concentration of 300 mcg/ml in 1 ml (300 mcg) and 1.6 ml (480 mcg) vials.

Preparation: For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water (DO NOT DILUTE WITH NORMAL SALINE) to a concentration greater than 5 mcg/ml. Filgrastim diluted to concentrations between 5 and 15 mcg/ml should be protected from absorption to plastic materials by the addition of albumin (human) to a final concentration of 2 mg/ml. When diluted in 5% dextrose or 5% dextrose plus albumin (human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes.

Storage and Stability: Filgrastim for injection should be stored in the refrigerator at 2° to 8°C (36° to 46°F). Avoid shaking.

Route of administration: Subcutaneous injection or intravenous infusion over 15–30 minutes.

Toxicities: Refer to section 16.3 Risks and Discomforts

18 REFERENCES

1. Primary cutaneous T cell lymphoma: Review and current concepts. JCO 2000; 18:2908
2. Diamandidou E Cohen PR, and Kurzrock R. Mycosis fungoides and Sezary Syndrome. Blood 1996; 88:2385
3. Weinstock MA, Horm J. Mycosis Fungoides in the United States: Increasing incidence and descriptive epidemiology. JAMA 1988; 260:42
4. Broder S, Edelson R, Lutzner M et al. The Sezary Syndrome: a malignant proliferation of helper T cells. J Clin Invest 1976; 58:1297
5. Edelson R, Berger C, Raafat G, Warburton D. Karyotype studies of cutaneous T cell lymphoma: evidence for clonal origin. J Invest Dermatol 1979; 73:548
6. Weiss LM, Hu E, Wood GS et al. Clonal rearrangements of T-cell receptor genes in mycosis fungoides and dermatopathic lymphadenopathy. N Engl J Med 1985; 313:539
7. Berger CL, Eisenberg A, Soper L et al. Dual genotype in cutaneous T cell lymphoma: immunoglobulin gene rearrangement in clonal T cell. J Invest Dermatol 1988; 90:73
8. Haynes BF, Metzgar RS, Minna JD, Bunn PA. Phenotypic characterization of cutaneous T cell lymphoma: use of monoclonal antibodies to compare with other malignant T cells. N Engl J Med 1981; 304:1319
9. Kung PC, Berger CL, Goldstein G et al. Cutaneous T cell lymphoma: characterization by monoclonal antibodies. Blood 1981; 304:1319
10. Harris NL, Jaffe ES, Stein H et al. A revised European American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994; 84:1361

11. Rook AH, Heald P. The immunopathogenesis of cutaneous T cell lymphoma. *Hematol Oncol Clin North Am* 1995; 9:997
12. Pickler LJ, Michie SA, Rott LS et al. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* 1990; 136:1053
13. Nakase K, Kitz K, Naser K et al. Differential expression of interleukin –2 receptors (alpha and beta chain) in mature lymphoid neoplasms. *Am J Hematol* 1994; 46:179
14. Hale G, Swirsky D, Waldmann H and Chan LC. Reactivity of rat monoclonal antibody CAMPATH-1 with human leukemia cells and its possible application for autologous bone marrow transplantation. *Br J Hematol* 1985; 60:41
15. Hale G, Xia MQ, Tighe HP et al. The CAMPATH-1 antigen (CDw52). *Tissue Antigens* 1990; 35:118
16. Foss FM, Sausville EA. Prognosis and staging of cutaneous T cell lymphoma. *Hematol Oncol Clin North Am* 1995; 9:1011
17. Minna JD, Roenigh HH, Glatstein E. Report of the committee on therapy for mycosis fungoides ad Sezary Syndrome. *Cancer Treat Rep* 1979; 63:729
18. Lamberg SI, Green SB, Byar DP et al. Status report of 376 mycosis fungoides patients at 4 years: Mycosis Fungoides Cooperative Group. *Cancer Treat Rep* 1979; 63:701
19. Sausville EA, Eddy JL, Makuch RW et al. Histopathologic staging at initial diagnosis of mycosis fungoides and Sezary syndrome. *Ann Int Med* 1988;109:372
20. Hoppe RT, Abel EA, Deneau DG et al. Mycosis fungoides: Management with topical nitrogen mustard. *JCO* 1987; 5:1796
21. Vonderheid EC, Tan ET, Kantor AF et al. Long- term efficacy, curative potential and carcinogenicity of topical meclorothamine chemotherapy in cutaneous T cell lymphoma. *J Am Acad Dermatol* 1989; 20:416
22. Reddy S, Parker CM, Shidnia H et al. Total skin electron beam therapy for mycosis fungoides. *Am J Clin Oncol* 1992; 15:119
23. Hoppe RT, Cox RS, Fuks Z et al. Electron beam therapy for mycosis fungoides: The Stanford University experience. *Cancer Treat Rep* 1979; 63:691
24. Quiros PA, Kacinski BM, Wilson LD. Extent of skin involvement as a rognostic indicator of disease free and overall survival in T3 stage cutaneous T cell lymphoma patients treated with total skin electron beam radiation therapy. *Cancer* 1996; 77:1912
25. Braverman IM, Yager NB, Chen M et al. Combined total body electron beam irradiation and chemotherapy for mycosis fungoides. *J Am Acad Dermatol* 1987;16:45
26. Wilson LD, Jones GW, Kim D et al. Experience with total skin electron beam therapy in combination with extracorporeal photopheresis in the management of erythrodermic mycosis fungoides. *J Am Acad Dermatol* 2000;43:54
27. Wilson LD, Licata AL, Braverman IM et al. Systemic chemotherapy and extracorporeal photopheresis for T3 and T4 cutaneous T-cell lymphoma patients who have achieved a complete remission to total skin electron beam therapy. *Int J Radiation Oncology* 1995; 32(4): 987-995
28. Hermann JJ, Roenigk HH, Hurria A et al. Treatment of mycosis fungoides with photochemotherapy (PUVA): Long term follow-up. *J Am Acad Dermatol* 1995;33:234
29. Rosen ST, Foss FM. Chemotherapy for mycosis fungoides and the Sezary syndrome. *Hematol Oncol Clin North Am* 1995;9:1109
30. Zackheim HS, Epstein EH. Low-dose methotrexate for the Sezary syndrome. *J Am Acad Dermatol* 1989; 21;757
31. Kuzel TM, Hurria A, samuelson E et al. Phase II trial of 2-chlorodeoxyadenosine for the treatment of cutaneous T-cell lymphoma. *Blood* 1996;87:906
32. Grever MR, Bisaccia E, Scarborough DA et al. An investigation of 2-deoxycoformycin in the treatment of cutaneous T cell lymphoma. *Blood* 1983; 61:279
33. Cummings FJ, Kim K, Neiman RS et al. Phase II trial of Pentostatin in refractory lymphomas and cutaneous T cell disease. *JCO* 1991; 9:565
34. Bunn PA, Ihde DC, Foon KA et al. The role of recombinant interferon alfa-2a in the therapy of cutaneous T cell lymphomas. *Cancer* 1986; 57:1689
35. Olsen EA, Rosen ST, Vollmer RT et al. Interferon alfa-2a in the therapy of cutaneous T cell lymphomas. *J Am Acad Dermatol* 1989; 20:395

36. Molin L, Thomsen K, Volden G et al. 13-cis-retinoic acid in mycosis fungoides. In Saurat JH (ed): *Retinoids: New trends in Research and Therapy*. Basel, Switzerland, Karger 1985;p341
37. Kuzel T, Olsen E, Martin A et al. Pivotal phase III trial of two dose levels of Dab 389 IL-2 (Ontak) for the treatment of mycosis fungoides. *JCO* 2001;19:376
38. Edelson R, Heald P, Perez M et al. Photopheresis update. *Prog Dermatol* 1991;25:1
39. Fraser-Andrews E, Seed P, Whittaker S et al. Extracorporeal photopheresis in Sezary syndrome: No significant effect in the survival of 44 patients with a peripheral blood T-cell clone. *Arch Dermatol* 1998; 134:1001
40. Foukaneli T, Marsh JCW, Pettegell R, et al. Sezary syndrome responds to treatment with Campath-1H. *Blood* 2001; 98(11): Abstract#556. p132a
41. Ferrajoli A, O'Brien SM, Williams ML et al. Campath-1H in refractory hematologic malignancies expressing CD52. A phase II clinical trial of 68 patients. *Blood* 2001; 98(11): Abstract # 1541. p366a
42. Lundin J, Osterborg A, Brittinger G et al. CAMPATH-1H monoclonal antibody in therapy of previously treated low-grade non-Hodgkin's lymphomas: a phase II multicenter study. European Study Group of CAMPATH-1H Treatment in low-grade non-Hodgkin's lymphoma. *JCO* 1998; 16:3257
43. Cooper DL, Braverman IM, Sarris AH, et al. Cyclosporine treatment of refractory T-cell lymphoma. *Cancer* 1993; 71:2335
44. Totterman TH, Scheynius A, Killander A et al. Treatment of therapy-resistant Sezary syndrome with cyclosporine A: suppression of pruritus, leukemic T-cell activation markers and tumor mass. *Scand J Hematol* 1985; 34: 196
45. Puttick L, Pollock A, Fairburn E. Treatment of Sezary syndrome with cyclosporine A. *JR Soc Med* 1983; 76:1063
46. Hoppe RT, Medeous LJ, Warnke RA et al. CD8 positive tumor infiltrating lymphocytes influence the long term survival of patients with mycosis fungoides. *J Am Acad Dermatol* 1995; 32:448
47. Vonderheid EC, Tan E, Sobel EL et al. Clinical implications of immunologic phenotyping in cutaneous T-cell lymphoma. *J Am Acad Dermatol* 1987; 17:40
48. Meissner K, Michaelis M, Rehpenning W et al. Epidermal Langerhans' cell densities influence survival in mycosis fungoides and Sezary syndrome. *Cancer* 1990; 65:2069
49. Marolleau JP, Baccard M, Flageul B et al. High-dose recombinant interleukin-2 in advanced cutaneous T-cell lymphoma. *Arch Dermatol* 1995; 131:574
50. Burt RK, Guitart J, Traynor A et al. Allogeneic hematopoietic stem cell transplantation for advanced mycosis fungoides: evidence of a graft versus tumor effect. *Bone Marrow Transplant*. 2000; 25:111
51. Molina A, Nademane A, Arber DA, Froman SJ. Remission of refractory Sezary syndrome after bone marrow transplantation from a matched unrelated donor. *Biol Blood Marrow Transplant* 1999;5:400
52. Molina A, Arber D, Murata-Collins JL et al. Clinical, cytogenetic and molecular remissions after allogeneic hematopoietic stem cell transplantation for refractory Sezary syndrome and tumor-stage mycosis fungoides. *Blood* 2001; 98(11): Abstract # 1715. p409a
53. Weiden P, Flournoy N, Thomas E et al. Antileukemic effects of graft-versus-host disease in human recipients of allogeneic marrow grafts. *New Eng J Med* 1979; 300:1068-1073.
54. Horowitz M, Gale RP, Sondel P, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*, 1990; 75: 552-562.
55. Apperley JF, Jones L, Hale G et al. Bone marrow transplantation for patients with chronic myeloid leukemia: T cell depletion with Campath-1 reduces the incidence of graft-versus-host disease but may increase the risk of leukemic relapse. *Bone Marrow Transplantation*, 1986; 1:53-68.
56. Goldman JM, Gale RP, Bortin MM et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase: increased risk of relapse associated with T-cell depletion. *Annals of Int Med*, 1988; 108:806-814.
57. Marmont AM, Horowitz MM, Gale RP et al. T cell depletion of HLA-identical transplants in leukemia. *Blood*, 1991; 78:2120-2130.
58. Kolb HJ, Mittermueller J, Clemm C et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990;
59. Heslop HE, Brenner MK, Rooney CM et al. Donor T cells to treat EBV-associated lymphoma. *N Engl J Med* 1994;331:679-680

60. Papadopoulos EB, Ladanyi M, Emanuel D et al. Infusions of donor leukocytes to treat EBV associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N Engl J Med* 1994;330:1185-1191
61. van Besien KW, de Lima M, Giralt SA et al. Management of lymphoma recurrence after allogeneic transplantation: The relevance of graft-versus-lymphoma effect. *Bone Marrow Transplant* 1997;19:977-982.
62. Verdonck L, Lokhorst H, Dekker A et al. Graft-versus-myeloma effect in 2 cases. *Lancet* 1996;347:800-801.
63. Tricot G, Vesole D, Jagannath S, Hilton J, Munshi N, Barlogie B. Graft-versus myeloma effect: proof of principle. *Blood* 1996, 87:1196-8.
64. Lokhorst HM, Schattenberg A, Cornelissen JJ, Thomas LLM, Verdonck LF. Donor leukocyte infusions are effective in relapsed multiple myeloma after allogeneic bone marrow transplantation. *Blood* 1997; 90:4206-4211
65. Jones RJ; Ambinder RF; Piantadosi S; Santos GW Evidence of a graft-versus lymphoma effect associated with allogeneic bone marrow transplantation. *Blood* 1991; 77: 649-53
66. Khouri IF, Keating M, Korbling M et al. Transplant-lite: Induction of graft versus malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *JCO*, 1998; 16:2817-2824.
67. Giralt S, Estey E, Albitar M et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft versus leukemia without myeloablative therapy. *Blood*, 1997; 89:4531-4536
68. Slavin S, Nagler A, Naparastak E et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood*, 1998; 91: 756-63
69. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med*. 2000 Sep 14;343(11):750-8.
70. Childs R, Clave E, Tisdale J et al. Successful treatment of metastatic renal cell carcinoma with a nonmyeloablative allogeneic peripheral blood progenitor cell transplant: Evidence for a graft-versus-tumor effect. *J Clin Oncol* 1999;17:2044-51.
71. Rini B, Zimmerman TM, Gajewski TF, et al. Allogeneic peripheral blood stem cell transplantation for metastatic renal cell carcinoma. *J Urol* 2001 Apr;165(3):1208-9
72. Reichman L, Clark M, Waldman H et al. Reshaping human antibodies for therapy. *Nature* 1988; 332:323
73. Treuman A, Lifely MR, Schneider P et al. Primary structure of CD52. *J Biol Chem* 1995; 270:6088
74. Gilleece MH, Dexter TM. Effect of Campath-1H antibody on human hematopoietic progenitors in vitro. *Blood* 1993; 82:807
75. Xia MQ, Hale G, Waldman H. Efficient complement-mediated lysis of cells containing the CAMPATH-1 (CDw52) antigen. *Mol Immunol* 1993; 30:1089
76. Matteson EL, Yocum DE, St. Clair EW et al. Treatment of active refractory rheumatoid arthritis with humanized monoclonal antibody CAMPATH-1H administered by daily subcutaneous injection. *Arthritis Rheum* 1995; 38:1187
77. Issacs JD, Watts RA, Hazleman BL et al. Humanized monoclonal antibody therapy for rheumatoid arthritis. *Lancet* 1992; 340:748
78. Osterborg A, Dyer MJ, Bunjes D et al. Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia: European Study Group of CAMPATH-1H treatment in chronic lymphocytic leukemia. *JCO* 1997; 15:1567
79. Osterborg A, Fassas AS, Anagnostopoulos A, et al. Humanized CD52 monoclonal antibody Campath –1J as first line treatment in chronic lymphocytic leukemia. *Br J Hematol* 1996; 93:151
80. Pawson R, Dyer MJ, Barge R et al. Treatment of T-cell prolymphocytic leukemia with human CD52 antibody. *JCO* 1997; 15:2667
81. Hale G, Dyer MJ, Clark MR et al. Remission induction in non-Hodgkin's lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* 1988; ii:1394
82. Hale G, Cobbold S, Waldman H. T-cell depletion with Campath-1 in allogeneic bone marrow transplantation. *Transplantation* 1988; 4445:753

83. Hale G, Waldman H. Recent results using Campath-1H antibodies to control GVHD and graft rejection. *Bone Marrow Transplant* 1996; 17:305
84. Kottaridis PD, Milligan DW, Chopra R et al. In-vivo CAMPATH-1H prevents graft versus host disease following nonmyeloablative a tem cell transplantation. *Blood* 2000; 96:2419
85. Flynn JM, and Byrd JC. Campath-1H monoclonal antibody therapy. *Curr Opin Oncol* 2000; 12:574
89. Jones GW, Hoppe RT, and Glatstein E. Electron Beam treatment for cutaneous T-Cell lymphoma. *Hem Onc Clin N America* 1995; 9(5): 1057-1076
90. Gehan, E. (1961). The determination a of the number of patients required in a preliminary and a follow-up trial of a new chemotherapeutic agent. *Journal of Chronic Diseases* 13: 346-353
91. Bartynski WS, Tan HP, Boardman JF, Shapiro R, Marsh JW. Posterior reversible encephalopathy syndrome after solid organ transplantation. *AJNR Am J Neuroradiol.* 2008 May;29(5):924-30. Epub 2008 Feb 13.)
92. Kopp HG, Horger M, Faul C, Hartmann JT, Kanz L, Lang P, Vogel W, Granulocyte colony-stimulating factor induced pulmonary hemorrhage in a healthy stem cell donor. Granulocyte colony-stimulating factor induced pulmonary hemorrhage in a healthy stem cell donor. *J Clin Oncol.* 2007 Jul 20; 25(21):3174-5)
93. Bacigalupo et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. *Hematologica* 2010.
94. Avivi et al. Matched unrelated donor allogeneic transplantation provides comparable long-term outcome to HLA-identical sibling transplantation in relapsed diffuse large B-cell lymphoma. *Bone marrow transplantation* 2014.
95. Wael Saber et al. Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia. *Blood.* 2012;119(17):3908-3916.
96. Duarte RF et al. Haematopoietic stem cell transplantation for patients with primary cutaneous T-cell lymphoma. *Bone Marrow Transplant.* 2008;41(7):597-604.
97. Molina A et al. Durable Clinical, Cytogenetic, and Molecular Remissions After Allogeneic Hematopoietic Cell Transplantation for Refractory Sezary Syndrome and Mycosis Fungoides. *J Clin Oncol.* 2005;23(25):6163-71.
98. Geller NL, Follmann D, Leifer ES, Carter SL, Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach. In *Advances in Clinical Trial Biostatistics* (ed. Geller NL), 2004. New York: Marcel Dekker, Inc.
99. Lechowicz et al. Allogeneic hematopoietic cell transplantation for mycosis fungoides and Sezary syndrome. *Bone Marrow Transplantation* (2014) 49, 1360–1365.
99. Duarte RF et al. Allogeneic Hematopoietic Cell Transplantation for Patients With Mycosis Fungoides and Se'zary Syndrome: A Retrospective Analysis of the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. *J Clin Oncol* 2010.
100. Duarte RF et al. Long-Term Outcome of Allogeneic Hematopoietic Cell Transplantation for Patients With Mycosis Fungoides and Se'zary Syndrome: A European Society for Blood and Marrow Transplantation Lymphoma Working Party Extended Analysis. *J Clin Oncol* 2014.
101. K.K. Reichard, Q.Y. Zhang, L. Sanchez, J. Hozier, D. Viswanatha, K. Foucar Acute myeloid leukemia of donor origin after allogeneic bone marrow transplantation for precursor T-cell acute lymphoblastic leukemia: case report and review of the literature *Am J Hematol*, 81 (2006), pp. 178–185
102. Z. Lang, P. Dinndorf, S. Ladisch, E. Bayever, G. Reaman Chromosomal transformation in donor cells following allogeneic bone marrow transplantation *Bone Marrow Transplant*, 33 (2004), pp. 1253–1256
103. L.D. Cooley, D.A. Sears, M.M. Udden, W.R. Harrison, K.R. Baker Donor cell leukemia: report of a case occurring 11 years after allogeneic bone marrow transplantation and review of the literature *Am J Hematol*, 63 (2000), pp. 46–53
104. Deitz, A et al. Donor-derived myelodysplastic syndrome and acute leukaemia after allogeneic haematopoietic stem cell transplantation: incidence, natural history and treatment response. *10.1111/British Journal of Hematology.* 12847

19 TABLES 1 and 2

TABLE 1. Long –term outcome by stage of 241 patients with Mycosis Fungoides treated with Total Skin Electron Beam Therapy at Stanford University (1958-1993), data based on Jones et al.[89]

Stage	#Patients	% Cutaneous CR	5 yr survival	10 year survival	15 year survival
IA	26	96	92	83	78
IB	52	56	75	57	46
IIA	63	63	71	56	33
IIB	46	24	35	18	7
III	42	26	46	37	17
IV	12	8	25	13	13

TABLE 2. Long –term outcome by stage of 320 patients with Mycosis Fungoides treated with Total Skin Electron Beam Therapy at Hamilton Clinic (1969-1993), data based on Jones et al.[89]

Stage	#Patients	% Cutaneous CR	5 yr survival	10 year survival	15 year survival
IA	103	84	91	87	74
IB	94	81	83	68	53
IIA	38	74	54	39	39
IIB	44	53	41	23	23
III	20	50	38	38	26
IVA	12	33	49	39	39
IVB	9	22	29	14	0

APPENDIX A TNM CLASSIFICATION OF CUTANEOUS T-CELL LYMPHOMA

Tumor (T)

T0	Clinically or histopathologically suspicious lesions
T1	Limited plaques, papules, or eczematous patches covering <10% of the skin surface
T2	Generalized plaques, papules, or erythematous patches covering >10% of the skin surface
T3	Tumors
T4	Generalized erythroderma

Lymph Nodes (N)

N0	No clinically abnormal peripheral lymph nodes, no pathological evidence of CTCL in any lymph node
N1	Clinically abnormal peripheral lymph nodes, pathology negative for CTCL
N2	No clinically abnormal peripheral lymph nodes, pathology positive for CTCL
N3	Clinically abnormal peripheral lymph nodes, pathology positive for CTCL

Visceral organ metastases (M)

M0	No visceral organ involvement
M1	Visceral organ involvement (must have pathology confirmation and organ involved should be specified)

Blood (B)*

B0	< 5% atypical (Sezary) lymphocytes in circulation
B1	>5% atypical (Sezary) lymphocytes in circulation

Stage Groupings

IA	T1, N0, M0
IB	T2, N0, M0
IIA	T1-2, N1, M0
IIB	T3, N0-1, M0
III	T4, N0-1, M0
IVA	T1-4, N2-3, M0
IVB	T1-4, N0-3, M1

* B classification not used in stage groupings

APPENDIX C PREPARATIVE REGIMEN

Day	Regimen
Day -28	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 3 mg over 1-2 hours
Day -27	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 10 mg over 1-2 hours
Day -26	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 30 mg over 1-2 hours
Day -24	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 30 mg over 1-2 hours
Day -22	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 30 mg over 1-2 hours
Day -19	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 30 mg over 1-2 hours
Day -17	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 30 mg over 1-2 hours
Day -15	Tylenol 650mg po
	Benadryl 50 mg IV
	Campath 1-H 30 mg over 1-2 hours
Day -5 to -1	Fludarabine 25 mg/ms/day IVPB over 30 minutes
	Antiemetics (phenothiazine per current NIH antiemetic guidelines)

APPENDIX D DONOR STEM CELL MOBILIZATION, AND APHERESIS

I. Donor stem cell mobilization with G-CSF and donor stem cell apheresis

After medical evaluation and clearance for suitability as an allogeneic donor, each donor will undergo mobilization with G-CSF as an outpatient. The donor will receive 10µg/kg G-CSF s.c. daily starting day -4 before apheresis. On day 0, a large volume leukapheresis of 15- 25 liters will be performed. The volume of the leukapheresis procedure will be targeted to obtain a progenitor cell dose of 5×10^6 CD34+ cells/kg. Obtaining a dose of $> 3 \times 10^6$ CD34+ cells/kg will be considered adequate and no further apheresis will be performed. If after the first day of apheresis, the product contains $< 3 \times 10^6$ /kg CD34+ cells, a decision regarding further apheresis will be made at the discretion of the PI, in consultation with Department of Transfusion Medicine. The minimum acceptable CD34+ cell dose to proceed with the protocol is 2×10^6 /kg.

Predictable side effects of G-CSF, including headache, bone pain, and myalgia, will be treated with acetaminophen or ibuprofen. Prophylactic treatment of these side effects may also be carried out with the same medications. Other side effects will be evaluated and treated accordingly.

Leukapheresis procedures will be carried out in the Apheresis Unit, Department of Transfusion Medicine. These procedures will be done via a 2-armed approach or by temporary central venous catheter. These procedures will use ACD-A anticoagulant, routinely used in normal donors. If the donor is very small or relatively intolerant to ACD-A, and the adverse effects cannot be controlled by usual means, consideration will be given to using partial anticoagulation with heparin.

II. Leukapheresis PBPC product

The processing goals for this protocol are to provide a product with a maximum dose of CD34+ cells. ABO or other erythrocyte incompatibility is not an issue because the resulting number of contaminating erythrocytes is well below 1 ml using this system. The final cell suspension will be prepared for infusion by transferring the cells into a 50-150 ml volume of an infusible solution (Plasmalyte A with 1% human serum albumin), suitable for intravenous infusion over 15 minutes to 1 hour, depending on clinical circumstances.

APPENDIX E 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 Allograft Transplantation 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 myelodysplastic 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 serum chemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramer staining).	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: <i>DKC1</i> , <i>TERC</i> , <i>TERT</i> , <i>SBDS</i> , <i>NOP10</i> , <i>NHP2</i> .	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 fluorescence 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 inoculation 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, circoviruses, 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 α 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 microdissection 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 membranous 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

