Amendment

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Protocol Title: Allogeneic HSCT Without Preparative Chemotherapy or With Low-Intensity Preparative Chemotherapy Using Sirolimus and Sirolimus Generated Donor Th2 Cells for Therapy of Refractory Leukemia, Lymphoma, Myeloma, or Myelodysplastic Syndrome

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

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

IRB Meeting Date: N/A

DEC Clearance Date: 09/28/2017

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B. Obtaining identifiable private information about living individuals
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Drug Name:	Th2 Cells in Allogeneic HSCT
IND Number:	11832
Sponsor:	Rapa Therapeutics
Manufacturer:	Cell Processing, Transfusion Medicine, CCR

Commercial Agents: Cyclophosphamide, Filgrastim, Cyclosporine, Sirolimus, Fludarabine, Etoposide, Doxorubicin, Vincristine, Rituximab, Prednisone

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PRÉCIS

Background

In protocol 99-C-0143, we evaluated a new approach to allogeneic HSCT that involved intensive host T cell ablation and graft augmentation with in vitro generated donor Th2 cells. Rapid full donor engraftment occurred with this regimen; however, grade II to IV acute GVHD was not significantly reduced in Th2 cell recipients. In an attempt to improve clinical results using Th2 cell graft engineering, this second-generation Th2 cell clinical trial was developed that incorporates the following interventions: (1) In an attempt to reduce transplant-related toxicity, this protocol now uses a very low-intensity host preparative chemotherapy; (2) In an attempt to reduce GVHD, this study will utilize Th2 cells expanded in the presence of the immune modulation agent, rapamycin (sirolimus), as murine Th2 cells grown in rapamycin reduce GVHD more effectively than control Th2 cells; (3) To further reduce GVHD, subjects will receive a short-course of sirolimus therapy in addition to standard cyclosporine GVHD prophylaxis; and (4) Using this novel low-intensity transplant platform, compare in a preliminary manner the post-transplant outcome of patients receiving pre-emptive donor lymphocyte infusion (DLI) using either Th2 cells or unmanipulated donor T cells.

Objectives

In the setting of HLA-matched sibling allogeneic HSCT using GVHD prophylaxis of cyclosporine and short-course sirolimus, compare in a preliminary manner the safety, feasibility, alloengraftment, clinical anti-tumor effects, and GVHD rate of low-intensity Preparative Chemotherapy with pre-emptive DLI using either Th2 cells or unmanipulated T cells at day 14 post-HSCT.

Eligibility

Subjects that are 16 to 75 years of age that have a suitable 6/6 HLA-matched sibling donor are potentially eligible. Subjects with a diagnosis of acute or chronic leukemia, non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, or myelodysplastic syndrome are potentially eligible. Adequate kidney, cardiac, and pulmonary function are required.

Design

- Patients age 18 or older with lymphoma (all types) or chronic lymphocytic leukemia will be randomized just prior to the transplant regimen to receive DLI with either donor Th2 cells (cohort 1) or unmanipulated T cells (cohort 2); n=10 patients will be accrued to each arm provided that stopping rules pertaining to excessive GVHD or graft rejection are not met. For these randomized patients, the preparative regimen will consist of low-intensity fludarabine (120 mg/m^2) plus cyclophosphamide (1200 mg/m^2) and GVHD prophylaxis will consist of short-course, high-dose sirolimus followed by maintenance cyclosporine. Cohorts 1 and 2 will be compared in a preliminary manner with respect to their post-transplant outcome, in particular: (a) conversion of mixed chimerism to predominant donor chimerism; (b) rate and severity of classical acute and late acute GVHD at the day 100 and day 180 post-transplant time points; and (c) time to induction of leukemia/lymphoma remission (if entering transplant with disease) or time to relapse (if entering transplant in remission).
- Patients with non-lymphoma diagnoses, patients with lymphoma that are under the age of 18 and lymphoma patients that are projected to be unable to complete the protocol-defined therapy through day 180 post-transplant will not be randomized but will be treated on cohort

3 (n=40), which will evaluate transplantation without the Flu/Cy preparative regimen and with pre-emptive Th2 cell DLI. The primary objective of cohort 3 is to evaluate whether transplantation without a preparative regimen will reduce the rate of acute GVHD associated with Th2 cell DLI from 41% (the rate observed with the Flu/Cy preparative regimen) to a rate of 15% (6 cases out of 40).

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Study Objectives

a) In the setting of HLA-matched sibling allogeneic HSCT using GVHD prophylaxis of cyclosporine (CSA) and short-course sirolimus (to day 14 post-HSCT), determine the safety, feasibility, alloengraftment, and GVHD rate of:
   1) Low-intensity Preparative Chemotherapy + 12-day cultured Th2 cells at day 14 post-HSCT (arm IVA).
   2) With Amendment P, a new primary study objective has been added due to addition of a new treatment arm, Arm IVB, which is evaluating Th2 cells that are expanded for 6-days in culture rather than the 12-days in culture that was utilized on prior Arm IV (now termed "IVA"). This new objective mirrors the objective that relates to arm IVA. That is, in the setting of HLA-matched sibling allogeneic HSCT using GVHD prophylaxis of cyclosporine (CSA) and short-course sirolimus (to day 14 post-HSCT), determine the safety, feasibility, alloengraftment, and GVHD rate of low-intensity Preparative Chemotherapy + 6-day cultured Th2 cells at day 14 post-HSCT (arm IVB).
   3) With amendment R, a new primary objective has been added that will compare the frequency of full donor lymphoid engraftment in patients treated on arms IVA and IVB. Specifically, we will test our hypothesis that recipients of Th2 cells manufactured in 6-days (arm IVB) will yield a higher frequency of full donor lymphoid engraftment relative to recipients of Th2 cells manufactured in 12-days (arm IVA).
   4) With amendment U, a new primary objective will be to determine the incidence of grade II to IV acute GVHD that occurs on a new arm IVC. Arm IVC therapy will be exactly the same as arm IVB therapy except that the GVHD prophylaxis regimen will be intensified through use of a higher steady-state target of sirolimus.
   5) With amendment AA, a new primary objective will be to compare in a preliminary manner the transplant outcome of lymphoma patients receiving low-intensity transplantation and pre-emptive DLI with either donor Th2 cells (arm IVD, cohort 1) or unmanipulated donor T cells (arm IVD, cohort 2).
   6) With amendment AA, for non-lymphoma patients and for lymphoma patients deemed to be not suitable for randomization, a new primary objective will be to evaluate the engraftment effects and GVHD profile of Th2 cell therapy in the absence of a Fludarabine/Cyclophosphamide preparative regimen (arm IVD, cohort 3).
   7) To clarify, with amendment AA, there will be no patient accrual to any of the other previous treatment cohorts (accrual will only be to arm IVD, cohorts 1, 2, and 3)
   8) With amendment CC, a new primary objective has been established for cohort 3: that is, to determine whether cohort 3 transplantation without a preparative regimen reduces the rate of acute GVHD associated with pre-emptive Th2 cell DLI from 41% to 15%.

1.1.2 Secondary Study Objectives

a) Characterize the pattern of post-transplant $CD4^+$ and $CD8^+$ T cell production of Th1- and Th2-type cytokines post-transplant.
b) Characterize the pattern of post-transplant $CD14^+$ monocyte production of inflammatory cytokines IL-1-α and TNF-α post-transplant.

c) For each treatment arm, characterize the kinetics of alloengraftment, the incidence of opportunistic infection, and the incidence of malignant disease complete remission.
d) With amendment R, a new secondary objective has been added. In a preliminary manner, we will compare arm IVA and IVB for their ability to result in complete remission in patients with malignancy at high risk for disease progression. Specifically, we will test our hypothesis that recipients of Th2 cells manufactured in 6-days (arm IVB) will yield a higher frequency of patients in complete remission relative to recipients of Th2 cells manufactured in 12-days (arm IVA).
e) In amendment AA, compare in a preliminary manner the ability of donor T-Rapa cells (arm IV, cohort A) and donor unmanipulated T cells (arm IV, cohort B) to achieve full donor chimerism (>95% donor) in the T cell and the myeloid cell lineages at days 28 and 100 post-transplant.

1.2 BACKGROUND AND RATIONALE

1.2.1 Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) represents a curative therapy for some patients with hematologic malignancy, including patients with acute and chronic leukemias, low-grade and high-grade non-Hodgkin's lymphoma, Hodgkin's Disease, and multiple myeloma(1). Initially, the curative component of allogeneic transplantation was attributed to myeloablative preparative regimens such as high-dose chemotherapy combined with high-dose total body irradiation. However, current evidence indicates that donor T cells contained within the allograft contribute significantly to the curative effect, which has been termed allogeneic graft-versus-leukemia or graft-versus-lymphoma (GVL) effect(2). Existence of such a GVL effect is supported by observations that malignant disease relapse is higher in recipients of T cell-depleted allografts(3), and that administration of donor T cells remote from the preparative regimen (donor lymphocyte infusion, DLI) can mediate tumor regression(4, 5).

Because of the contribution of this immune-based mechanism to anti-tumor effects post-transplant, efforts have been initiated to reduce the intensity of the preparative regimen, as myeloablative preparative regimens are associated with a significant degree of morbidity and mortality(6). Such efforts, which can be termed "reduced-intensity" HSCT, involve administration of immune depleting agents prior to transplant for the purpose of preventing allograft rejection. Unfortunately, reduced-intensity HSCT is still associated with most of the major complications associated with myeloablative transplantation, including graft rejection, toxicity from immune suppression medications, acute and chronic GVHD, opportunistic infection, and malignant disease relapse(7-10). Therefore, reduced intensity transplantation, although it appears to beneficially reduce toxicity associated with the transplant preparative regimen, is still limited by donor anti-host immune reactions (GVHD and insufficient GVL effects) and host anti-donor immune reactions (graft rejection).

1.2.2 Immunoablative Reduced Intensity Allogeneic HSCT (Protocol 99-C-0143)

In an attempt to improve upon results with reduced intensity HSCT, we initiated NCI protocol 99-C-0143, which consisted of an "immunoablative" form of reduced intensity transplantation. In prior reduced intensity trials, there was a significant incidence of graft rejection, and a particularly high incidence of mixed donor/host chimerism post-transplant. The occurrence of mixed chimerism appears, in general, not to be highly clinically beneficial, as it can lose stability

and convert to full host chimerism (graft rejection), convert to full donor chimerism with subsequent onset of GVHD, and delay the onset of GVL effects(11-14). In a murine graft rejection model, we found that non-radiation based, immune depleting chemotherapy that resulted in quantitative host T cell depletion comparable to levels obtained with high dose total body irradiation were required for efficient prevention of graft rejection in the reduced-intensity context(15). In light of these findings, we developed an immunoablative transplant regimen, (protocol 99-C-0143) with the specific aim of consistent abrogation of graft rejection and mixed chimerism. This regimen included administration of outpatient immune-depleting EPOCH/fludarabine chemotherapy, with the goal of reducing host $CD4^+$ and $CD8^+$ T cells. This approach was successful, as median host $CD4^+$ and $CD8^+$ T cell numbers were reduced from study entry to completion of EPOCH-F (n=48 subjects: CD4 cells reduced from 283 to 97 cells/ul; CD8 cells reduced from 261 to 52 cells/ul).

After EPOCH/F, patients then received transplant preparative chemotherapy that consisted of total doses of fludarabine and cyclophosphamide that were similar to other reduced-intensity protocols(12); however, based on our murine data that these two agents depleted immune cells in a synergistic manner(15), we elected to administer these agents concomitantly, not in a sequential manner utilized in other protocols. After the preparative regimen, host immunity was indeed relatively ablated, with levels of host $CD4^+$ and $CD8^+$ T cells that were close to the detection limit of the flow cytometry assay (<3 and < 1 cells/ul, respectively). Consistent with our hypothesis, transplantation after host immune depletion resulted in rapid donor alloengraftment, with recovery of neutrophils > 500 cells/ul and platelets > 20,000 cells/ul at a median of 8 and 10 days post-transplant. Mixed chimerism was consistently abrogated, as 47/47 recipients had complete donor T cell and myeloid cell elements by day 28 post-transplant.

As we have recently published(16), immunoablative reduced-intensity transplantation was associated with significant GVL effects, which, in somewhat of a contrast to other reduced intensity approaches, occurred rapidly within the first 100 days post-transplant, developed independent of GVHD prophylaxis withdrawal, and was observed in patients with bulky and highly-chemotherapy refractory malignancy. However, acute GVHD remained a major complication of this transplant approach, with 63% (12/19) of individuals developing grade II-IV acute GVHD. This incidence and severity of acute GVHD is similar to that previously observed with myeloablative transplantation using single agent cyclosporine GVHD prophylaxis(17), and is similar to recent results using myeloablative or reduced-intensity transplantation in patients with a relatively advanced median age (~ 50 years of age)(18).

1.2.3 Th2 Cell Augmentation of Allogeneic HSCT: Results from Pilot Study 99-C-0143

Because donor T cells initiate both beneficial GVL effects and detrimental GVHD after allogeneic transplantation, efforts are underway to identify T cell therapy strategies that can mediate anti-tumor effects with reduced GVHD. Such strategies include the administration of specific doses of T cells, $CD4^+$ or $CD8^+$ enriched T cells, T cells depleted of the alloreactive repertoire, or T cells with suicide gene insertion(19-23). As an alternative, we have evaluated whether functionally defined T cell subsets of $CD4^+$ Th1/Th2(24) or $CD8^+$ Tc1/Tc2(25, 26) cytokine phenotype might differentially influence allogeneic transplantation responses. In murine models, we and others found that murine $CD4^+$ T cells of Th2-type and $CD8^+$ T cells of Tc2-type resulted in less GVHD relative to their Th1 and Tc1 counterparts(27, 28); importantly, such Th2/Tc2 strategies could be harnessed to allow for beneficial transplantation responses such

as the mediation of GVL effects and the abrogation of rejection concomitant with reduced GVHD(29-32).

In an initial attempt to clinically translate these findings, we evaluated in protocol 99-C-0143 whether a strategy of Th2 cell augmentation of allogeneic HSCT might allow for a beneficial GVL effect while limiting acute GVHD. Because we identified that murine Th2 cells did not themselves mediate a significant GVL effect but could modulate unmanipulated donor $CD4^+$ and $CD8^+$ T cells, we elected to administer Th2 cells in the context of a T cell replete allograft. To generate Th2 cells for this clinical trial, we modified a clinically relevant method of in vitro T cell expansion based upon CD3, CD28 co-stimulation that has been utilized for adoptive T cell therapy trials in the setting of HIV infection(33) and post-autologous transplant immune reconstitution(34). Specifically, donor $CD4^+$ T cells were isolated, in vitro activated on day 0 and 12 of culture with anti-CD3, anti-CD28 coated magnetic beads in the presence of IL-2 and the Th2-promoting cytokine, IL-4(35). At the end of the 20 day culture interval, the donor CD4 cells displayed an effector Th2 phenotype, as evidenced by the generation of CD4 cells that were largely $CD28^-$, $CD62L^-$, CD40 $ligand^+$. Relative to cells at initiation of culture, Th2 cells had a loss of IL-2 secretion, a reduction in IFN-γ secretion, and an increase in secretion of the Th2 cytokines IL-4, and in particular, IL-13(36).

Accrual to the Th2 therapy arms of protocol 99-C-0143 has been completed, and all protocol patients are more than 100 days post-HSCT and being followed for long-term protocol endpoints of chronic GVHD and status of malignancy. Demographics of subjects treated on the Th2 treatment arms are shown here in Table I (n=28 subjects). As this table shows, the median age of Th2 recipients was 48.5 years (range, 25 to 69 years). This protocol cohort was therefore advanced in age relative to the majority of allogeneic transplantation series reported, and because of the known association of advance recipient age with increased GVHD, was likely at increased risk for transplant-related morbidity and mortality.

Table I. Th2 Recipients: Patient Accrual and Demographics

Subject UPN	On-Study	Age	Gender	Race[1]	Th2 Level/PBSCT Date
Th2-UPN-01	10/19/00	62	Male	CAU	Th2 Level #1 (1/5/01)
Th2-UPN-02	11/20/00	48	Female	CAU	Th2 Level #1 (1/29/01)
Th2-UPN-03	12/21/00	69	Male	CAU	Th2 Level #1 (2/8/01)
Th2-UPN-04	1/11/01	49	Male	CAU	Th2 Level #2 (4/5/01)
Th2-UPN-05	2/08/01	43	Female	CAU	Th2 Level #2 (3/19/01)
Th2-UPN-06	2/08/01	38	Female	CAU	Th2 Level #2 (4/12/01)
Th2-UPN-07	3/08/01	46	Male	CAU	Off-Study Prior to SCT
Th2-UPN-08	3/21/01	25	Female	HSP	Th2 Level #2 (5/31/01)
Th2-UPN-09	8/24/01	37	Female	HSP	Th2 Level #2 (11/27/01)
Th2-UPN-10	9/17/01	45	Male	CAU	Th2 Level #2 (10/24/01)
Th2-UPN-11	10/02/01	31	Female	CAU	Th2 Level #3 (1/02/02)
Th2-UPN-12	12/12/01	45	Male	CAU	Off-Study Prior to SCT
Th2-UPN-13	1/15/02	50	Male	CAU	Th2 Level #3 (4/01/02)
Th2-UPN-14	1/15/02	45	Male	CAU	Th2 Level #3 (4/02/02)
Th2-UPN-15	2/06/02	54	Female	CAU	Th2 Level #3 (4/23/02)
Th2-UPN-16	2/14/02	53	Male	af am	Th2 Level #3 (5/15/02)
Th2-UPN-17	3/19/02	36	Male	Asian	Th2 Level #3 (6/03/02)
(Following patients: accrued to expanded Phase II Cohort, Th2 Dose Level #2)					
Th2-UPN-18	3/19/02	44	Male	CAU	Th2 Level #2 (6/10/02)
Th2-UPN-19	4/09/02	30	Male	CAU	Th2 Level #2 (7/01/02)
Th2-UPN-20	4/30/02	60	Male	af am	Th2 Level #2 (7/15/02)
Th2-UPN-21	5/8/02	54	Male	CAU	(died prior to transplant)
Th2-UPN-22	5/14/02	48	Male	CAU	(did not receive Th2 cells)
Th2-UPN-23	5/21/02	58	Female	af am	Th2 Level #2 (5/24/02)
Th2-UPN-24	5/29/02	26	Male	CAU	Th2 Level #2 (7/24/02)
Th2-UPN-25	5/30/02	50	Male	CAU	Th2 Level #2 (7/23/02)
Th2-UPN-26	6/26/02	57	Male	CAU	Th2 Level #2 (7/29/02)
Th2-UPN-27	7/15/02	36	Male	CAU	Th2 Level #2 (8/21/02)
Th2-UPN-28	7/16/02	57	Male	CAU	Th2 Level #2 (10/16/03)
Th2-UPN-29	7/19/02	51	Female	CAU	Th2 Level #2 (10/7/02)
Th2-UPN-30	7/30/02	62	Male	Asian	Off-Study Prior to SCT
Th2-UPN-31	7/31/02	53	Female	CAU	Th2 Level #2 (10/29/02)
Th2-UPN-32	8/16/02	48	Male	CAU	Th2 Level #2 (10/15/02)
Th2-UPN-33	9/10/02	54	Male	CAU	Th2 Level #2 (11/25/02)

[1] Abbreviations: CAU = Caucasian; HSP = Hispanic; af am = African American

Allograft augmentation with in vitro activated Th2 cells was well tolerated, with no acute toxicity attributable to the Th2 cells at doses of 5, 25, or 125 x 10^6 Th2 cells/kg. As Table II below shows, alloengraftment was rapid in Th2 recipients. Median time to neutrophil recovery of > 500 cells/ul was 9 days (range, 7 to 13 days), and median time to platelet recovery > 20,000 cells/ul was 10 days (range 8 to 12 days). Prior studies of reduced-intensity allogeneic HSCT have been associated with a risk of graft rejection and typically have demonstrated mixed donor/host chimerism. In contrast to these prior results, allogeneic HSCT with additional donor Th2 cells was not associated with graft rejection, and there was not a single case of mixed chimerism at the relatively early time point of day 14 post-transplant (0/28 cases). As such, the rapid and complete nature of alloengraftment in Th2 recipients is more similar to the pattern observed with HSCT utilizing myeloablative preparative regimens(37, 38). Relative to non-Th2 recipients, Th2 recipients had an early increase in $CD4^+$ and $CD8^+$ T cells, an increase in both Th1- and Th2-type cytokines post-transplant, and an increase in monocyte inflammatory cytokine production post-transplant(39). These results indicate that Th2 cells activated donor immunity in the presence of post-transplant cyclosporine administration, with contribution from both the activated Th2 cells and the unmanipulated $CD4^+$ and $CD8^+$ T cells contained within the stem cell allograft. In light of our murine data that type I immunity is associated with enhanced anti-tumor responses after allogeneic transplantation(40), this mixed pattern of type I/type II immunity may represent a favorable pattern of post-transplant immune activation for mediation of GVL and GVT effects.

Table III. Th2 Recipients: Alloengraftment

Subject UPN	Th2 Level	Hematopoietic Recovery		Donor Chimerism[3]
		Neutrophil[1]	Platelet[2]	
Th2-01	# 1	13	12	100%
Th2-02	# 1	9	12	100%
Th2-03	# 1	8	11	100%
Th2-04	# 2	10	DIC	100%
Th2-05	# 2	10	10	100%
Th2-06	# 2	9	11	100%
Th2-08	# 2	9	10	90%
Th2-09	# 2	10	12	99%
Th2-10	# 2	9	10	100%
Th2-11	# 3	9	11	100%
Th2-13	# 3	9	10	100%
Th2-14	# 3	9	10	85%
Th2-15	# 3	9	11	100%
Th2-16	# 3	7	8	85%
Th2-17	# 3	9	9	99%
Th2-18	# 2	8	9	98%
Th2-19	# 2	9	11	95%
Th2-20	# 2	8	10	100%
Th2-23	# 2	9	11	95%
Th2-24	# 2	9	10	100%
Th2-25	# 2	10	11	95%
Th2-26	# 2	8	9	85%
Th2-27	# 2	9	10	100%
Th2-28	# 2	9	10	99%
Th2-29	# 2	9	8	100%
Th2-31	# 2	8	11	100%
Th2 32	# 2	8	10	95%
Th2 33	# 2	10	10	100%

[1] Neutrophil recovery (day post-SCT) as defined by ANC > 500 cells/ul.
[2] Platelet recovery (day post-SCT) as defined by platelet count > 20,000 cells/ul; "DIC" indicates patient remained platelet transfusion dependent due to disseminated intravascular coagulation.
[3] Donor chimerism was determined by VNTR-PCR analysis on whole blood mononuclear cells collected at day 14 post-SCT.

Allogeneic HSCT with Th2 cells was associated with significant anti-tumor responses in the majority of recipients, most of whom had highly refractory hematologic malignancy at the time of study entry. Table III below details the diagnosis and prior therapy for each of the 28 patients receiving allogeneic SCT with Th2 cells on this study. The following diagnoses were represented: non-Hodgkin's lymphoma (n=15); multiple myeloma (n=5); Hodgkin's lymphoma (n=3); chronic lymphocytic leukemia (n=3); and accelerated phase chronic myelogenous

leukemia (n=2). As Table III shows, in general, subjects transplanted on this protocol were heavily pre-treated (median number of prior therapies of n=3, range 1 to 5). As this table details, 27/28 of these recipients had measurable malignant disease upon study entry, and in 20/28 cases, the malignant disease was refractory to conventional therapy. The majority of patients were further demonstrated to be refractory to the relatively aggressive induction chemotherapy regimen administered on this protocol (EPOCH-fludarabine): of evaluable patients receiving this regimen prior to allogeneic transplantation, 8/27 had progressive disease, 7/27 had stable disease, 11/27 had a partial remission, and 1/27 had a complete remission. In this relatively refractory patient population, allogeneic transplantation was associated with significant anti-tumor effects: 1/27 Th2 recipients had a minor response post-SCT (25 to 50% tumor decrease), 6/27 patients entered into a PR post-SCT, and 20/27 (74%) of patients entered into a CR post-SCT. This high complete response rate, which occurred in a patient population demonstrated to be generally refractory to conventional chemotherapy regimens and to experimental EPOCH-fludarabine administered on this protocol during induction therapy, suggests that allogeneic transplantation with Th2 cells is associated with a potent anti-tumor effect. Significantly, most of the complete responses obtained post-SCT have been durable. Although these results can most likely be attributed to an immune mediated graft-versus-leukemia effect, it is also possible that the fludarabine and cyclophosphamide preparative regimen also contributed significantly to the anti-tumor effects observed.

Table III: Th2 Recipients: Overall Transplantation Outcome (as of 06/01/03)

		Malignant Disease (Study Entry)				
Subject UPN	Th2 Level	Disease[1]	Prior Trx[2]	Disease Status[3]	Post-SCT Response[4]	Tumor Outcome[5]
Th2-01	# 1	CLL/Richter's	5	PR	CR	CR
Th2-02	# 1	Myeloma	2	1° Refractory	CR/PD	PD
Th2-03	# 1	Mantle Cell NHL	1	PR	CR	CR
Th2-04	# 2	Follicular NHL	4	1° Refractory	CR	CR
Th2-05	# 2	Large Cell NHL	2	MRD	NE-MRD	CR
Th2-06	# 2	LYG NHL/HPS	6	Refractory	PR	PD
Th2-08	# 2	CML	1	Acc. Phase	CR Mol.	CR Mol.
Th2-09	# 2	Follicular NHL	2	1° Refractory	CRu	CRu
Th2-10	# 2	Large Cell NHL	3-au	Refractory	CR	CR
Th2-11	# 3	Large Cell NHL	3-au	1° Refractory	CR	CR
Th2-13	# 3	Myeloma	1	PR	CR/PD	pending
Th2-14	# 3	CML	2	Acc. Phase	Heme R	PD
Th2-15	# 3	Large Cell NHL	2	Refractory	PR	PR
Th2-16	# 3	Myeloma	1	1° Refractory	CR	CR
Th2-17	# 3	Myeloma	1	1° Refractory	CR/PD	pending
Th2-18	# 2	Large Cell NHL	2-au	1° Refractory	CR	CR
Th2-19	# 2	Hodgkin's	3-au	Refractory	CR	CR
Th2-20	# 2	CLL/Richter's	4	Refractory	PR	CRu
Th2-23	# 2	CLL	4	Refractory	PR	PR
Th2-24	# 2	Hodgkin's	3-au	1° Refractory	CRu/PD	CR
Th2-25	# 2	Large Cell NHL	4	Refractory	CRu	CR
Th2-26	# 2	Follicular NHL	4	1° Refractory	MR/PD	PD
Th2-27	# 2	Hodgkin's	3-au	PR	PR/PD	Pending
Th2-28	#2	Large Cell NHL	2	PR	CR	CR
Th2-29	#2	Follicular NHL	3	Refractory	Cru	CRu
Th2-31	#2	Myeloma	3	PR	CR	CR
Th2-32	#2	Follicular NHL	2	Refractory	CR/PD	pending
Th2-33	#2	Follicular NHL	3	PR	Cru	CRu

[1] CLL (chronic lymphocytic leukemia); myeloma (multiple myeloma); NHL = non-Hodgkin's lymphoma; Richter's (Richter's high-grade lymphoma transformation); LYG/HPS (lymphomatoid granulomatosis with hemophagocytic syndrome).
[2] Number of chemotherapy regimens prior to study entry; "au" = prior autologous SCT.
[3] "PR" = partial response to chemotherapy prior to study entry; "1° refractory" = primary refractory disease (patient never achieved a partial response to chemotherapy); "NE-MRD" = not evaluable (had minimal residual disease); "Acc. Phase" indicates accelerated phase CML.
[4] Post-SCT Response indicates best anti-tumor response obtained. PR/PD indicates initial response followed by progressive disease (PD). CR mol. = molecular CR; MR = minor response.
[5] For tumor outcome, "pending" indicates that post-transplant therapy of donor lymphocyte infusion or chemotherapy has been administered, and response to this therapy is currently under evaluation.

Allogeneic HSCT with Th2 cells, although beneficially associated with rapid alloengraftment and significant anti-tumor effects, was limited by acute GVHD, which was the primary transplant complication contributing to morbidity and mortality. Th2 subjects, who received up to 1.25 x 10^8 CD28 co-stimulated donor CD4 cells/kg, had increased donor lymphocyte numbers and increased type I and type II cytokines post-transplant. This information indicates that the immunologic graft-versus-host reaction (GVHR) was actually increased in Th2 recipients. In this setting of increased GVHR, it is noteworthy that the cohort of Th2 recipients did not have increased clinical GVHD relative to the initial cohort not receiving Th2 cells. In fact, as detailed below in Table IV, the percentage of Th2 recipients without any acute GVHD (10/28; 36%) was higher relative to non-Th2 recipients (3/19; 16%). Nonetheless, the incidence of grade II-IV acute GVHD in Th2 recipients was 18/28 (64%), which was similar to the 12/19 incidence (63%) observed in the non-Th2 cohort. These incidences of grade II to IV acute GVHD, with or without Th2 cells, are comparable to those reported in a younger patient population treated with single agent cyclosporine as GVHD prophylaxis after full intensity myeloablative preparative regimens(17), or to similarly age-matched patients received two-drug GVHD prophylaxis after either myeloablative or reduced-intensity conditioning(18).

Our finding that additional CD28-activated donor Th2 cells can be administered with a T cell replete allograft without increased clinical GVHD indicates that a Th2 pattern of immune activation may be desirable after clinical allogeneic transplantation. It is possible that Th2 cell promotion of both type I and type II immunity insufficient cytokine polarization of the Th2 cell product, and to this extent, we reasoned that further improvement in this strategy might be obtained through the generation of Th2 cells with increased type II polarization (that is, reduced expression of the type I cytokine molecules, such as IL-2, IFN-γ, and CD40 ligand). In addition, we reasoned that alternative immune suppression drugs might promote Th2 biology to a greater degree than cyclosporine, which has been shown to inhibit both Th1 and Th2 cytokines(41).

Table IV: Th2 Recipients: Graft-Versus-Host Disease and Overall Survival

Subject UPN	Th2 Level	Acute GVHD[1] Day of Onset	Organ Staging	Grade	Survival Status	Cause of Death[2]
Th2-01	# 1	35	3-3-0	III	Died (day 141)	Infection/GVHD
Th2-02	# 1	78	3-0-0	II	Died (day 143)	Progressive Disease
Th2-03	# 1	28	3-1-0	II	Died (day 608)	Cardiovascular
Th2-04	# 2	17	3-0-0	II	Died (day 22)	DIC/Shock
Th2-05	# 2	N.A.	0-0-0	0	Alive (day 804)	N.A.
Th2-06	# 2	N.A.	0-0-0	0	Died (day 315)	PD/DLI-GVHD
Th2-08	# 2	N.A.	0-0-0	0	Alive (day 731)	N.A.
Th2-09	# 2	92	0-0-3	III	Alive (day 551)	N.A.
Th2-10	# 2	N.A.	0-0-0	0	Alive (day 585)	N.A.
Th2-11	# 3	N.A.	0-0-0	0	Alive (day 515)	N.A.
Th2-13	# 3	29	3-1-0	II	Alive (day 427)	N.A.
Th2-14	# 3	10	2-4-1	IV	Died (day 132)	PD/GVHD
Th2-15	# 3	N.A.	0-0-0	0	Died (day 339)	Infection/GVHD
Th2-16	# 3	28	0-2-0	II	Alive (day 382)	N.A.
Th2-17	# 3	16	2-2-0	II	Alive (day 363)	N.A.
Th2-18	# 2	32	0-0-4	IV	Died (day 125)	Infection
Th2-19	# 2	N.A.	0-0-0	0	Alive (day 336)	N.A.
Th2-20	# 2	26	0-4-0	IV	Died (day 56)	GI Bleed/GVHD
Th2-23	# 2	36	0-4-0	IV	Died (day 77)	Encephalopathy/GVHD
Th2-24	# 2	N.A.	0-0-0	0	Alive (day 312)	N.A.
Th2-25	# 2	27	0-3-0	III	Died (day 216)	Hem/Infection/GVHD
Th2-26	# 2	28	3-0-0	II	Died (day 99)	Progressive Disease
Th2-27	# 2	57	0-1-0	II	Alive (day 284)	N.A.
Th2-28	# 2	55	0-1-0	II	Alive (day 226)	N.A.
Th2-29	# 2	24	0-1-0	II	Alive (day 235)	N.A.
Th2-31	# 2	N.A.	0-0-0	0	Alive (day 213)	N.A.
Th2-32	# 2	24	3-2-0	III	Alive (day 227)	N.A.
Th2-33	# 2	N.A.	0-0-0	0	Alive (day 186)	N.A.

[1] Day of onset of acute GVHD is noted. Acute GVHD, measured through day 100 post-HSCT, was graded according to the modified Glucksberg Criteria (Overall Grade 0 to IV). Skin, intestine, and liver target organs were staged with scale of 0 to 4. Abbreviations: "NE" = not evaluable; "NA" = not applicable.
[2] "GVHD" = active GVHD or intensive immune suppression therapy of GVHD; "DIC" = disseminated intravascular coagulation; "DLI-GVHD" = GVHD after donor lymphocyte infusion; "Hem" = diffuse hemorrhage; "Encephalopathy" = severe cyclosporine A neurotoxicity.

1.2.4 Rapamycin Generated Th2 Cells: Murine Studies

In recent murine studies, we have evaluated whether specific immune suppression agents differentially modulate Th1 vs. Th2 biology. In these studies, which involved a CD3, CD28 system analogous to the clinical method utilized, we found that the calcineurin inhibitors cyclosporine and FK506 similarly inhibited the generation of both Th1 and Th2 cell differentiation. However, rapamycin, which modulates T cell function through inhibition of mTOR(42), the mammalian target of rapamycin, did not significantly inhibit Th2 cell generation. As shown in Figure 1, murine Th2 cells generated in rapamycin, in particular, high-dose rapamycin, exhibited an improved Th2 cytokine phenotype, as defined by reduced expression of

the Th1-type cytokines IL-2 and IFN-γ. Such rapamycin generated Th2 cells maintained secretion of the afferent Th2 cytokines IL-4 and IL-5, yet had reduced secretion of the type II cytokines IL-10 and IL-13 that are associated with more distal Th2 cell effector function. In addition, relative to control Th2 cells, rapamycin generated Th2 cells had reduced expression of surface CD40 ligand, a molecule associated with Th1 function via induction of IL-12 secretion from antigen-presenting-cell populations(43).

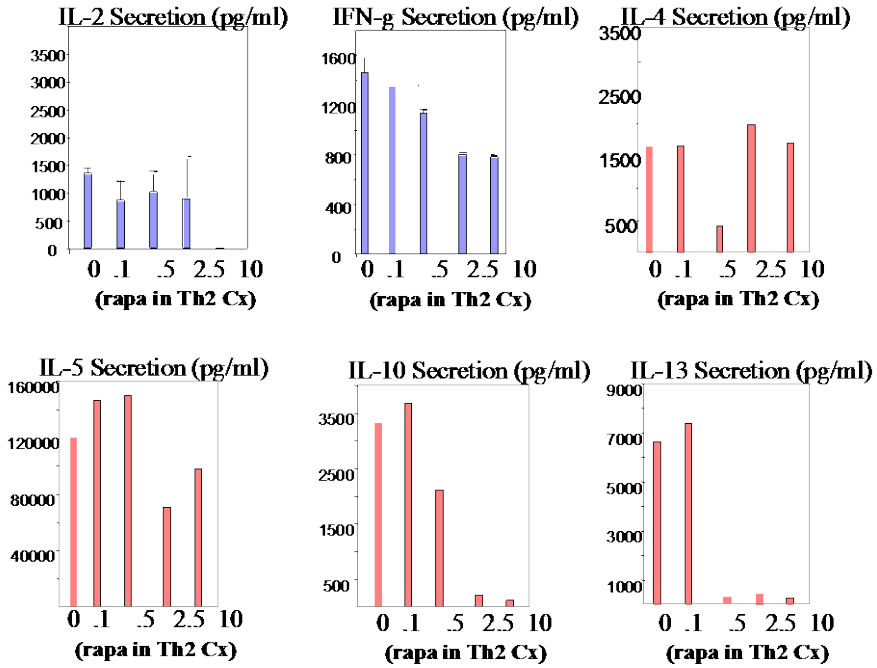


Figure 1: Rapamycin Enhances Murine Th2 Cell Generation. Murine splenic CD4+ T cells from C57Bl/6 mice were isolated and stimulated with anti-CD3, anti-CD28 coated magnetic beads in the presence of Th2 promoting cytokine conditions (+IL-4). Cultures were expanded withoutapamycin ("0") or in the presence of rapamycin ("0.1, 0.5, 2.5, or 10 micromolar"). On day 6 of culture, CD4 cells were harvested, brought to a concentration of 0.5 x 10⁶ cells/ml, and restimulated with anti-CD3, anti-CD28. The 24 hour supernatant was then tested for content of type I cytokines (IL-2, IFNγ) or type II cytokines (IL-4, IL-5, IL-10, and IL-13).

In a murine transplantation model that, analogous to the clinical trial design, involved Th2 cell augmentation of $CD4^+$ and $CD8^+$ T cell replete allografts, rapamycin generated Th2 cells reduced GVHD to a more significant degree than control Th2 cells generated in the absence of rapamycin. Relative to recipients of conventional Th2 cells, recipients of Th2 cells expanded in the presence of rapamycin had reduced IFN-γ secretion emanating from the Th2 population, and from $CD4^+$ and $CD8^+$ T cells contained within the allograft. Recipients of rapamycin generated Th2 cells also had reduced GVHD-related weight loss post-transplant and reduction in histologic lesions of GVHD, in particular, an absence of gut GVHD in mice receiving Th2 cell generated in high dose rapamycin (10 uM) (Figure 2).

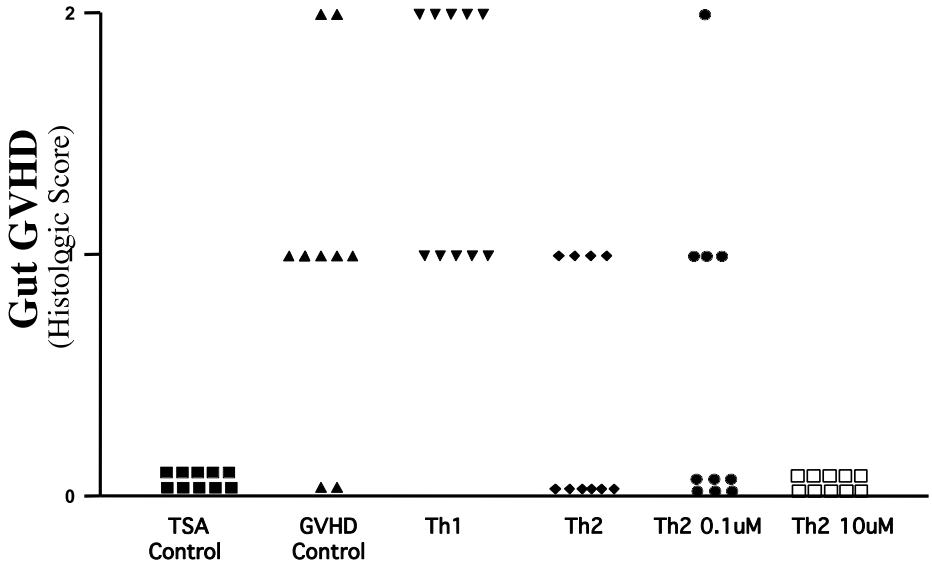
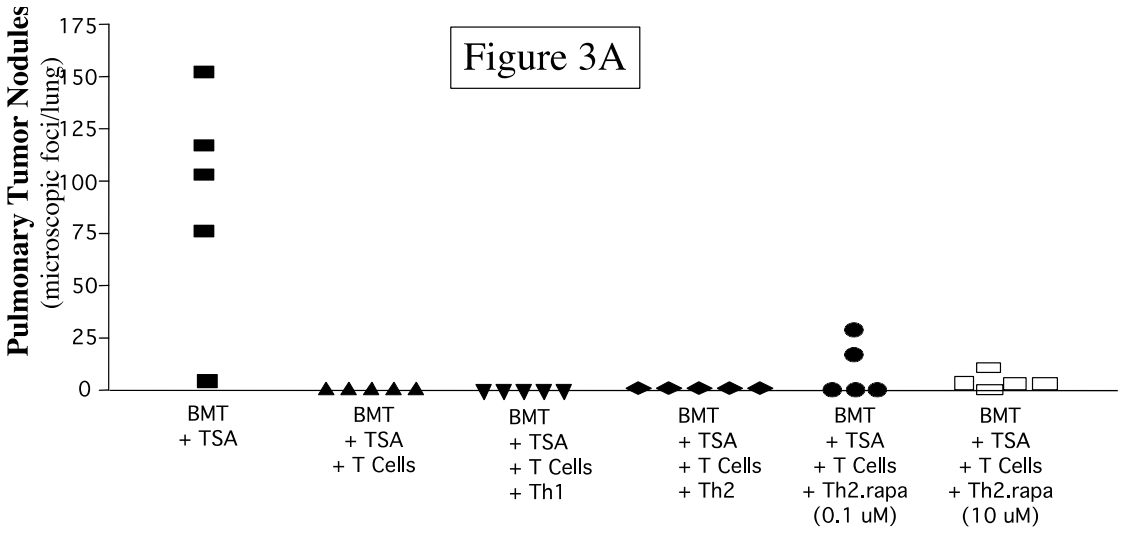
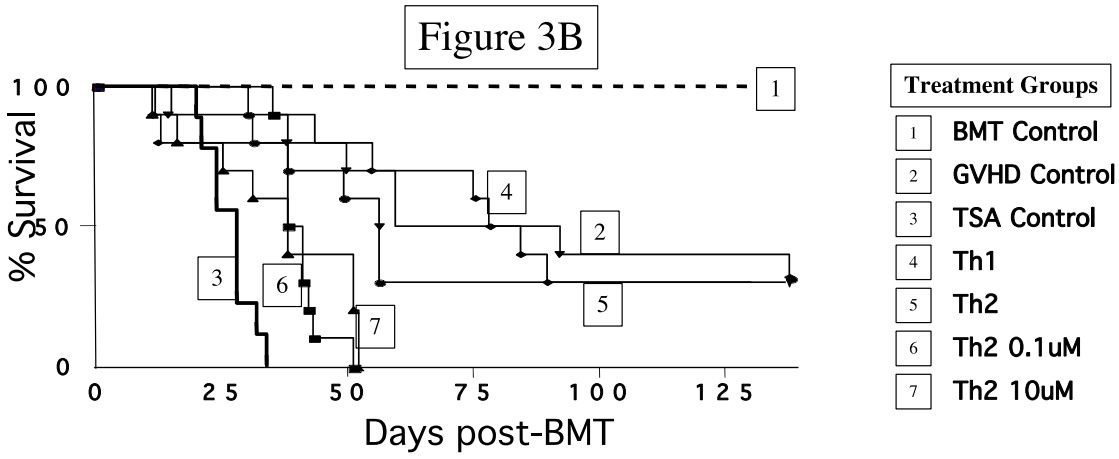


Figure 2. Rapamycin-generated Th2 cells have increased capacity to prevent murine GVHD. Lethally irradiated CB6F1 mice received bone marrow transplantation (BMT) from C57Bl/6 donors; each recipient also received 1 x 10^5 host-type breast cancer cells (i.v.; TSA cell line). The "TSA Control" group received no other inocula, whereas each of the other groups received additional donor splenic T cells. The "GVHD Control" group received only the splenic T cells, whereas the other recipients also received additional donor in vitro expanded CD4 cells generated in IL-12 ("Th1 group"), in IL-4 ("Th2 group"), in IL-4 and low-dose rapamycin (0.1 uM; "Th2 0.1 uM"), or in IL-4 and high-dose rapamycin (10 uM; "Th2 10 uM"). On day 14 post-BMT, GVHD target tissues were harvested and evaluated for histologic signs of acute GVHD. Stage of small intestinal and large intestinal acute GVHD in the treatment cohorts are shown (n=5 recipients in each cohort; 5 data points for small intestine, 5 data points for large intestine).

This transplant model also involved inoculation of recipient mice with host-type breast cancer cells (TSA cell line), which we have recently demonstrated to be primarily susceptible to a GVT effect mediated by donor cells of type I cytokine phenotype(40). As shown below in Figure 3, histology studies demonstrated that recipients of T cell replete allografts augmented with rapamycin generated Th2 cells had partial preservation of an allogeneic GVT effect. That is, pulmonary tumor foci (day 14 post-BMT) were reduced relative to the tumor control group that did not receive allogeneic T cells; recipients of rapamycin generated Th2 cells also had increased survival relative to this tumor control group (median survival of n = 10 recipients per group: 27.0 ± 1.4 d [TSA Control Treatment Group] vs. 40.5 ± 1.4 d [+ Th2 0.1 uM Rapamycin Treatment Group] vs. 36.5 ± 4.9 d [+Th2 10 uM Rapamycin Treatment Group]; each comparison, $p < 0.05$).





Administration of allogeneic T cells either alone or with additional Th1 or Th2 cells (non-

Figure 3. Allograft augmentation with rapamycin-generated Th2 cells partially preserves an allogeneic GVT effect. Lethally irradiated CB6F1 hosts inoculated with host-type breast cancer cells (TSA cell line; i.v.) received allogeneic BMT alone ("BMT + TSA") or with additional donor splenic T cells ("BMT + TSA + T cells"). Other groups received donor splenic T cells and additional donor in vitro generated CD4 cells that were expanded in the presence of IL-12 ("+ Th1"), IL-4 ("+ Th2"), IL-4 and low-dose rapamycin ("+ Th2.rapa [0.1 uM]), or IL-4 + high-dose rapamycin ("+ Th2.rapa [10 uM]"). On day 14 post-BMT, lungs were harvested, stained with H and E, and the number of microscopic pulmonary tumor foci were enumerated (Figure 3A). Post-BMT survival curves are shown in Figure 3B (n=10 per treatment group). Death in recipients of the rapamycin-generated Th2 cells was attributable to tumor, whereas death in the other T cell groups was attributable to lethal GVHD.

rapamycin generated) resulted in a potent GVT effect, as evidenced by lack of day 14 pulmonary tumor foci (tumor absent in 15/15 recipients); however, this potent GVT effect was highly associated with lethal GVHD, as death by GVHD through day 100 post-BMT was observed in 6/10 recipients ("GVHD Controls"; additional donor splenic T cells), 7/10 recipients ("+ Th1";

additional donor T cells and additional Th1 cells), and 7/10 recipients ("+ Th2"; additional donor T cells and additional non-rapamycin generated Th2 cells).

As such, rapamycin generated murine Th2 cells modulate type I cytokine-driven allogeneic responses to a significantly greater degree than conventional Th2 cells, as evidenced by greater reduction in type I cytokines after allogeneic transplantation, abrogation of type I cytokine-associated gut GVHD, and partial abrogation of type I cytokine-driven GVT effects. These murine results predict that, relative to the Th2 cells utilized on protocol 99-C-0143, sirolimus generated human Th2 cells will more effectively prevent GVHD but be less effective with respect to GVT effects. To address this potential outcome (abrogation of GVHD concurrent with an insufficient GVT effect), the protocol will allow for additional post-transplant anti-tumor interventions, including early discontinuation of cyclosporine GVHD prophylaxis, donor lymphocyte infusion (DLI), and chemotherapy administration.

In addition to usage of rapamycin in vitro for enhanced generation of Th2 cells, we have evaluated whether in vivo rapamycin administration post-transplant might enhance Th2 cell modulation of type I allogeneic immunity. As shown in Figure 4, the combination of Th2.rapa cells and in vivo rapamycin resulted in a greater number of Th2 cells post-transplant relative to in vivo cyclosporine or vehicle control administration. Significantly, recipients of Th2.rapa cells combined with in vivo rapamycin had a greater reduction in post-transplant levels of the type I cytokine IFN-γ relative to recipients of Th2.rapa cells combined with in vivo cyclosporine A. This result indicates that both in vivo rapamycin and in vivo cyclosporine can cooperate with Th2.rapa cells to reduce GVHD, with rapamycin administration being somewhat more effective. This result also indicates that a short-course of rapamycin in vivo might be utilized to increase the post-transplant number of the therapeutic Th2.rapa cells, with subsequent enhancement of their efficacy. These results are consistent with prior murine experiments that identified a shift towards type II immunity in recipients of post-transplant rapamycin(44).

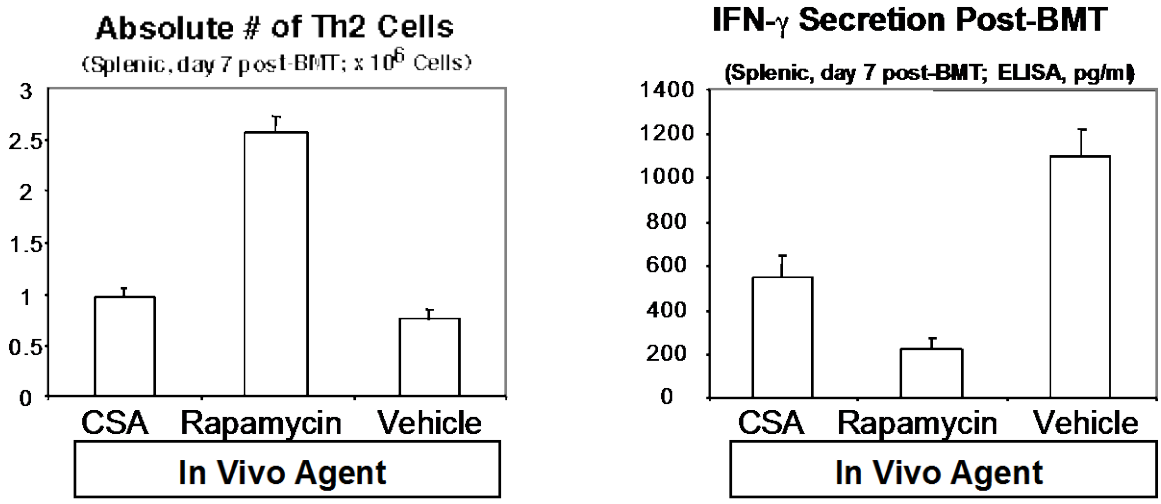


Figure 4. In vivo rapamycin for potentiation of murine Th2 cell prevention of GVHD. Murine donor C57Bl/6 bone marrow and splenic T cells (2 x 10^7 T cells) were injected into lethally irradiated CB6F1 hosts for GVHD induction. Mice received additional donor $CD4^+$ T cells of Th2 cytokine phenotype that were generated in the presence of rapamycin (1 x 10^7 Th2 cells). Post-BMT, recipients were treated with either cyclosporine A or rapamycin (each at 1.5 mg/kg/day) or vehicle control (n=5 per transplant group). Spleens were harvested on day 7 post-BMT, flow cytometry for the Ly5.1 congenic marker expressed on the expanded Th2 cells was performed, and the number of Th2 cells present in vivo was calculated (left panel). In the right panel, splenic T cells (day 7 post-BMT), were stimulated with anti-CD3 and anti-CD28, and the 24 hour supernatant was tested for content of the type I cytokine IFN-γ.

1.2.5 Sirolimus (Rapamycin) Generated Human Th2 Cells: Improved Th2 Polarization

In light of these murine results, we initiated pre-clinical studies to evaluate whether the commercially available form of rapamycin, sirolimus, might facilitate the generation of human $CD4^+$ T cells of enhanced Th2 purity. For these studies, we utilized the exact Th2 cell generation methodology used for protocol 99-C-0143, with the exception that the clinically approved agent sirolimus was added at culture initiation and maintained throughout the culture interval. For ease of subsequent protocol implementation, sirolimus was purchased in the formulation utilized for clinical therapy (Rapamune oral solution; Wyeth-Ayerst Pharmaceuticals). In this methodology, allogeneic transplant donor $CD4^+$ T cells are isolated by negative selection, stimulated with anti-CD3 and anti-CD28 monoclonal antibodies conjugated to magnetic beads, and expanded in X-Vivo 20 media supplemented with 5% donor plasma, recombinant human IL-4 (1000 I.U./ml), and recombinant human IL-2 (20 I.U./ml).

As Figure 5 below shows, addition of sirolimus (1 uM) to the Th2 cell generation method established for protocol 99-C-0143 was permissive for human CD4 cell expansion. Our murine experiments indicated that usage of high concentrations of rapamycin improved the anti-GVHD effect of the in vitro generated Th2 cells; as such, in the human studies utilizing the co-stimulation method of expansion, we first identified the highest concentration of sirolimus permissive for Th2 cell generation (1 uM). It should be noted that this in vitro concentration of

sirolimus is 100-fold greater than the concentration of sirolimus that can be safely maintained in vivo (10 ng/ml). As the top panel shows, sirolimus reduced peak human CD4 cell median cell volume under these Th2 culture conditions, as measured by Coulter Multisizer analysis. Median T cell volume after CD28 co-stimulation has been previously shown to correlate with T cell activation(45), and as such, sirolimus moderation of Th2 cell volume is consistent with a major effect of this drug, which is to inhibit mTOR mediated mRNA translation(42). As the bottom panel of Figure 5 shows, Th2 cell expansion in sirolimus was a highly selective culture condition. That is, the majority of normal donor CD4 cells did not expand in the presence of sirolimus, as reflected by an early reduction in CD4 cell number in the sirolimus condition. However, at later points in the Th2 cell expansion, sirolimus generated cells had a similar expansion profile relative to the control Th2 condition. This result indicates that the CD4 cells emanating from the Th2 condition containing sirolimus were relatively resistant to the inhibitory effects of sirolimus. Assuming a CD4 cell input number of 1 x 10^8 cells, which is easily attainable from transplant donor apheresis, CD4 cell expansion in the presence of sirolimus for 12 to 20 days in culture will allow administration of sirolimus generated Th2 cells at the dose established on protocol 99-C-0143, 2.5 x 10^7 Th2 cells/kg.

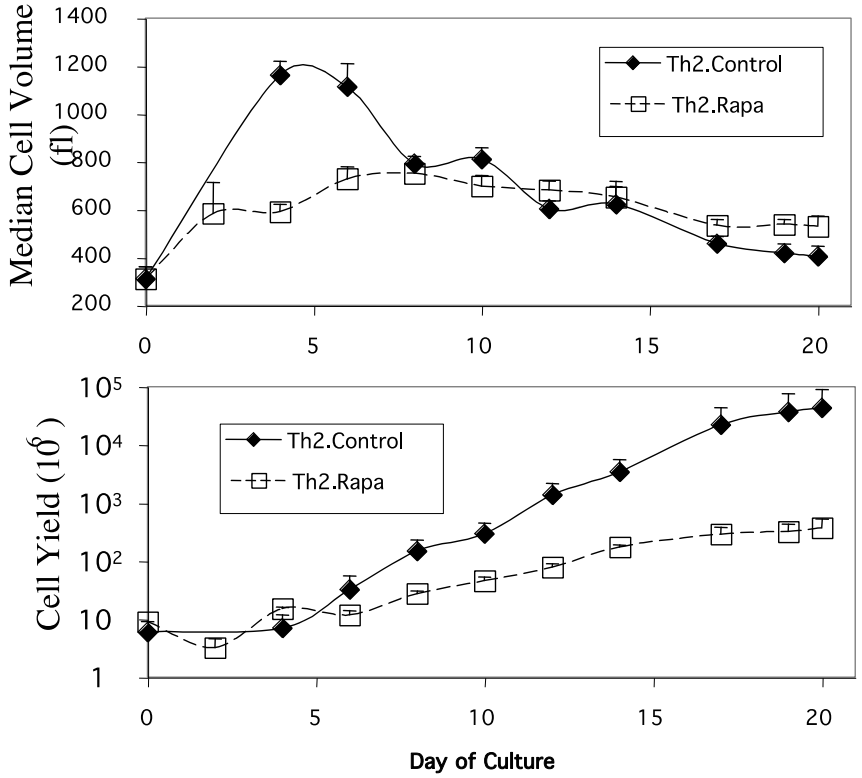


Figure 5: Rapamycin generated human Th2 cells: CD4 cell expansion, activation.
$CD4^+$ T-cells from four healthy donors were stimulated with anti-CD3/anti-CD28 coated beads on day 0, and grown in the presence of rhuIL-4 (1000 IU/ml) and rhuIL-2 (20 IU/ml) either alone (Th2.Control) or in the presence of rapamycin (1 uM; Th2.Rapa). CD4 cell activation was monitored by median cell volume determination (Coulter Multisizer). Results shown are mean ± SEM of n=4 Th2 expansion cultures.

To evaluate the effect of sirolimus on the cytokine profile of human Th2 cells, CD4 cells were harvested at day 12 or day 20 after culture initiation, and evaluated for their secretion of type I vs. type II cytokines. As Figure 6 below shows, CD4 cells propagated under Th2 conditions in the presence of sirolimus, but not in the absence of sirolimus, constitutively secreted the type II cytokine IL-4 at both day 12 and day 20 of culture. Upon CD3, CD28 re-stimulation, Th2 cells generated with or without sirolimus had a similar capacity for IL-4 secretion. As such, sirolimus increased Th2 cell constitutive IL-4 secretion and maintained full Th2 cell potential for IL-4 secretion. Because secretion of IL-4 is the primary mechanism whereby Th2 cells influence Th0 => Th2 and Tc0 => Tc2 differentiation(46, 47), this result suggests that, relative to control Th2 cells, sirolimus generated Th2 cells would be at least equal, and perhaps superior, in their capacity to promote type II immunity post-transplant.

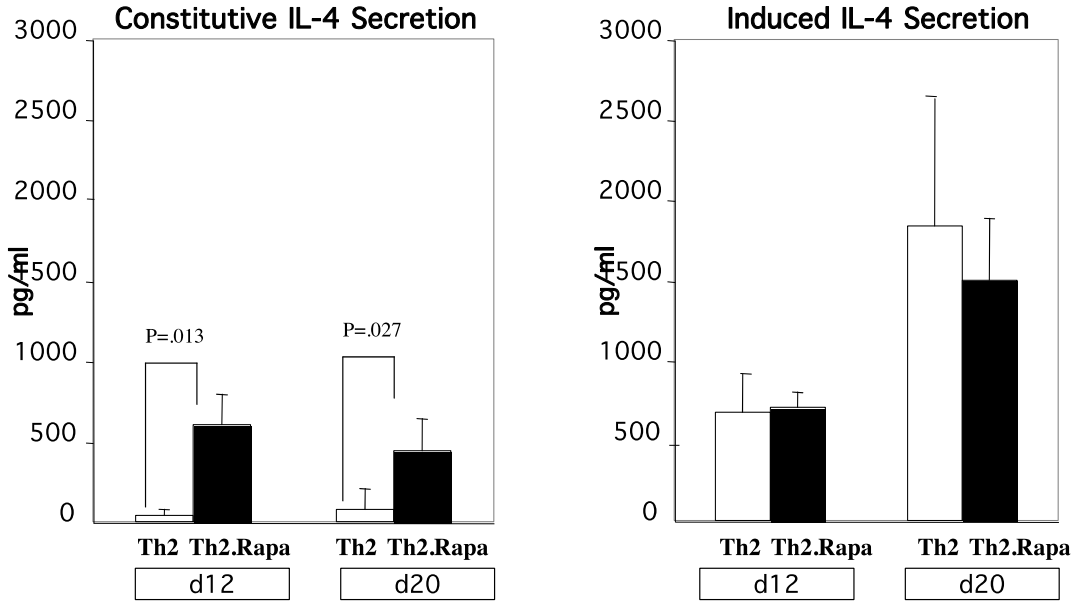


Figure 6. Th2 generation in rapamycin: IL-4 secretion. Human CD4+ T cells were isolated (n=4 donors), and stimulated with anti-CD3, anti-CD28 coated magnetic beads in the presence of rhu IL-4 (1000 IU/ml) and rhu IL-2 (20 IU/ml) either in the absence or presence of rapamycin (1 uM). Cells were expanded for 12 or 20 days, at which time CD4 cells were harvested, washed, and brought to a final concentration of 0.5 x 106 CD4 cells/ml. A 24 hour supernatant was generated under conditions of no stimulation (left panel; "constitutive") or anti-CD3, anti-CD28 re-stimulation (right panel: "induced"). The supernatants from each of the cultures was then tested for IL-4 content by ELISA, with the mean and SEM of n=4 cultures shown.

Most importantly, expansion of human CD4 cells in the Th2 promoting condition with sirolimus greatly improved the purity of Th2 cells, as reflected by a significant reduction in contamination with the Th1-type cytokines IL-2 and IFN-γ. As figure 7 below shows, the capacity for IL-2 secretion within the Th2 cultures was completely abrogated by sirolimus at both day 12 and day 20 of culture. As such, by as early as day 12 in culture, sirolimus generated Th2 cells have a higher propensity for secretion of IL-4 and abrogation of IL-2 secretion with a single round of co-stimulation. This cytokine secretion pattern may be particularly favorable with respect to regulation of GVHD, as prior studies have shown that the frequency of IL-4 secreting donor CD4 cells associates with reduced GVHD(48) and that the frequency of IL-2 secreting donor CD4

cells associates with increased GVHD(49). As figure 7 also demonstrates, secretion of the type I cytokine IFN-γ was also reduced in the sirolimus containing Th2 condition.

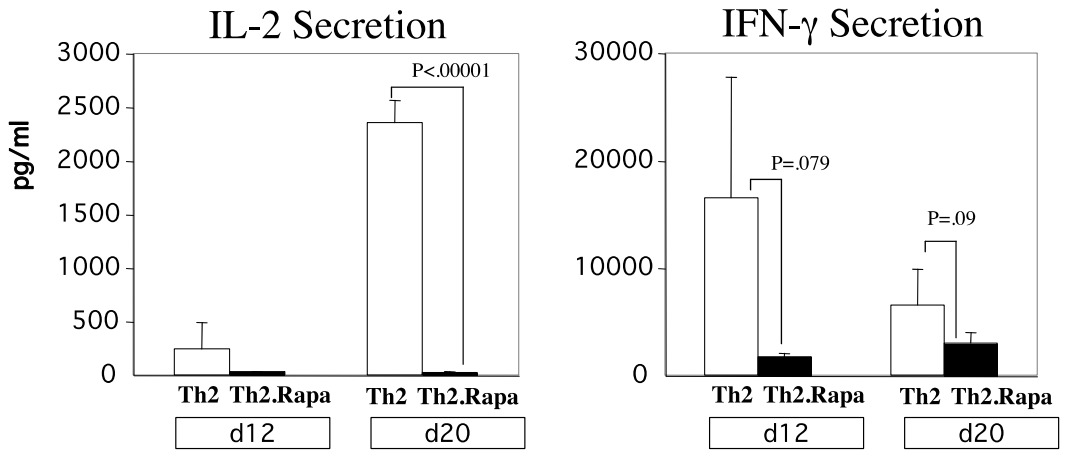


Figure 7: Rapamycin improves purity of Th2 cell generation: reduction in IL-2 and IFN-γ secretion.
CD4+ T cells from healthy adult donors (n=4) were stimulated with anti-CD3, anti-CD28 coated magnetic beads in the presence of rhu IL-4 (1000 IU/ml) and rhu IL-2 (20 IU/ml) either in the absence or presence of rapamycin (1 uM). On day 12 and day 20 of culture, CD4 cells were harvested, washed, adjusted to a final concentration of 0.5 x 10^6 CD4 cells/ml, and re-stimulated with anti-CD3, anti-CD28; the 24 hour supernatant was then tested for content of IL-2 and IFN-g by two-site ELISA assay. For the IL-2 assay, IL-2 secretion in the Th2.rapa condition was below the detection limits of the assay (31 pg/ml). Results shown are the mean and SEM of four cultures. Th2.Rapa cells produced 99% less IL-2 than control cells (p<.001).

Similar to our murine results (see Figure 1), addition of sirolimus to the Th2 culture condition resulted in human $CD4^+$ T cells that secreted reduced levels of the effector type II cytokines IL-5, IL-10, and IL-13. As Figure 8 below shows, relative to the non-sirolimus Th2 condition, sirolimus-generated human Th2 cells had reduced secretion of each of these cytokines at the earlier time point of evaluation, day 12 of culture. By day 20 of culture, however, IL-5 and IL-13 secretion capacity of the sirolimus generated Th2 cells was not reduced. Addition of sirolimus to the Th2 culture condition therefore promotes the generation of afferent Th2 cells that initially secrete high levels of the afferent Th2 cytokine IL-4, with subsequent acquisition of an effector Th2 phenotype.

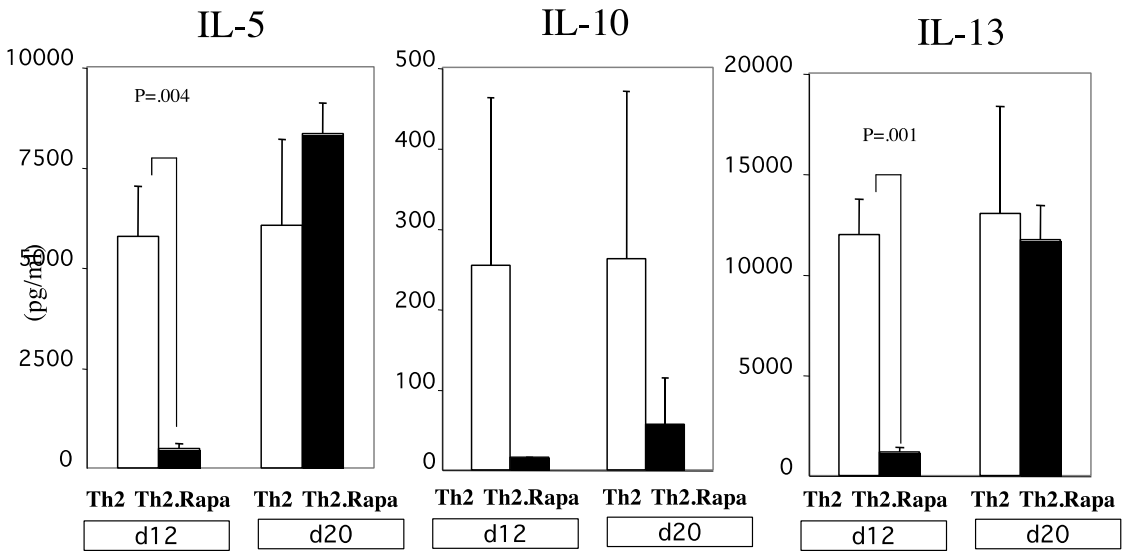


Figure 8: Rapamycin generated Th2 cells: afferent T-helper cytokine secretion profile. Human CD4+ T cells were isolated, stimulated with anti-CD3, anti-CD28 coated beads, and propagated in Th2 promoting conditions of rhu IL-4 (1000 IU/ml) and rhu IL-2 (20 IU/ml) in the absence or presence of rapamycin (1 uM). On day 12 and day 20 of culture, CD4 cells were harvested, washed, adjusted to a final concentration of 0.5 x 106 cells/ml, and restimulated with anti-CD3, anti-CD28. The 24 hour supernatant was then tested for content of the more distal effector Th2 cytokines, IL-5, IL-10, and IL-13. Results shown are the mean and SEM of n=4 cultures.

1.2.6 Sirolimus Administration After Allogeneic HSCT

Cyclosporine, which inhibits T cell function through calcineurin inhibition and subsequent down-regulation of T cell mRNA transcription, has been the primary immune suppression molecule utilized for GVHD prevention. However, GVHD prophylaxis using cyclosporine or the related molecule FK506, which also operates via a calcineurin inhibition mechanism, is still associated with significant GVHD (grade II-IV) in the majority of patients (~66%) receiving HLA-matched sibling transplantation(17). To this end, prior investigations have evaluated the role of additional immune suppression molecules, such as methotrexate and corticosteroids. In general, such two-agent GVHD prophylaxis can reduce the incidence and severity of GVHD in the myeloablative HSCT setting, although intensified immune suppression can delay alloengraftment, add significant toxicity, and may reduce donor T cell-mediated anti-tumor effects. Because of the inherent limitation of intensified GVHD prophylaxis (toxicity, reduced immunity), we are evaluating whether an alternative strategy, modulation of donor T cell cytokine phenotype towards a Th2 pattern, might be utilized to reduce GVHD. If such a cell modulation intervention were successful, the reliance upon chemotherapeutic immune suppression would predictably be reduced, or even eliminated.

More recently, the immune suppression drug sirolimus (rapamycin), which is effective in the prevention of renal allograft rejection(50), has been evaluated after allogeneic HSCT, initially as a therapy for chronic GVHD(51), and more recently, as a third agent for GVHD prophylaxis after genetically disparate transplantation(52). Sirolimus uniquely inhibits T cell and dendritic cell function through inhibition of mTOR(53), which in contrast to the mRNA inhibition associated with calcineurin blockade, operates via a post-transcriptional mechanism to reduce cell signaling and modulate protein translation and phosphorylation. Clinical studies have not evaluated single-agent sirolimus for GVHD prophylaxis, and in fact, murine data indicate that single-agent sirolimus potently abrogates $CD8^+$ T cell mediated GVHD, but not $CD4^+$ T cell

mediated GVHD(44). As such, sirolimus is likely to represent an adjunct to, but not a replacement of, calcineurin inhibitor mediated GVHD prophylaxis. However, use of sirolimus as a second GVHD prophylaxis agent may have significant negative aspects, as long-term sirolimus administration can cause significant toxicity (hypertriglyceridemia, pancreatitis, neutropenia, thrombocytopenia) and global immune suppression with associated opportunistic infection(54-57).

Initial results indicate that sirolimus is indeed an effective agent for upfront GVHD prophylaxis(58). In this single arm study, which involved unrelated donor or mis-matched family member bone marrow transplantation, GVHD prophylaxis involved calcineurin blockade (FK506), methotrexate, and sirolimus. Sirolimus was initiated at a loading dose of 12 mg, with a maintenance dose of 4 mg per day throughout the first 100 days post-transplant. The grade II-IV GVHD incidence associated with this regimen was 26% (11/41), which is reduced relative to historical controls. Sirolimus was also reported to be well-tolerated at this dosing schedule, with hypertriglyceridemia representing the primary specific drug toxicity.

In this protocol, we will evaluate whether short-course sirolimus administration (through day 14 post-SCT) in the peri-transplant period might alone improve CSA GVHD prophylaxis, or enhance Th2 cell prevention of GVHD. Evaluation of this approach is based in part upon our observation that, compared to freshly isolated T cells, sirolimus generated Th2 cells are relatively resistant to the growth inhibitory effects of further sirolimus exposure (see Figures 4 and 5). As such, we hypothesize that short-course in vivo sirolimus will preferentially inhibit unmanipulated donor T cells contained within the allograft, thereby resulting in preferential in vivo expansion of sirolimus generated Th2 cells. In the event that this preferential in vivo selection of the administered Th2 product were to occur, the type I/type II post-transplant cytokine balance would favor a Th2 pattern, with a predicted subsequent reduction in GVHD. Our usage of short-course sirolimus for potential optimization of Th2 cell therapy may define a novel use of this drug, with minimization of its associated immune suppression, toxicity, and significant interaction with other drugs such as cyclosporine. In an attempt to minimize these potential adverse effects of sirolimus, the drug will be discontinued after day +14 post-transplant. Because of the relatively long elimination half-life of sirolimus (62 hours), immune modulating drug levels may persist past the second week post-transplant. It is also possible that sirolimus alone (without Th2 cells) may contribute to GVHD prophylaxis. For example, in recent murine allogeneic bone marrow transplantation experiments, we have found that short-course (five day) sirolimus administration in the peri-transplant period significantly reduced fully-MHC mismatched graft-versus-host reaction. If short-course sirolimus alone were found to contribute to clinical GVHD prophylaxis, such a result would be analogous to short-course methotrexate administration, which is effective as a second agent for GVHD prophylaxis after myeloablative transplantation.

1.2.7 Overview of Protocol Treatment Arms and Arm IV, Arm V Rationale

Arms I, II, and III of the protocol, which are no longer open to accrual, utilized a “reduced-intensity” approach to allogeneic hematopoietic stem cell transplantation. The preparative regimen in these initial treatment arms consisted of fludarabine in combination with cyclophosphamide (total cyclophosphamide dose, 4800 mg/m^2). In arms I, II, and III, there was significant toxicity in the form of engraftment syndrome. In particular, engraftment syndrome was particularly problematic on arms I and II, which involved administration of the experimental donor Th2 cell product. As such, it was determined, in consultation with the FDA reviewers of

the IND file for the Th2 cell product (BB-IND 11832), that the initial Flu/Cy regimen was not a suitable platform for evaluating the Th2 cell therapy.

In murine experiments, we have subsequently determined that donor Th2 cells generated in rapamycin are particularly potent in terms of preventing graft rejection and promoting alloengraftment [results recently published: Mariotti et al, Journal of Immunology, 2008; 180(1): 89-105]. In light of this information, we reasoned that the donor Th2 cells may have promoted engraftment, thereby predisposing to engraftment syndrome. By corollary, we reasoned that because the primary purpose of the transplant preparative regimen is to ensure alloengraftment, that donor Th2 cell administration may allow transplantation with a lower intensity Flu/Cy preparative regimen or no Flu/Cy preparative regimen. To address this, the protocol was amended to initiate two new protocol arms, IV and V. Subjects are assigned to protocol arm IV vs. arm V based upon their $CD4^+$ T cell count. For patients who have a CD4 count less than 200 cells/µl, the patient is assigned to arm V and receives the transplant after the next dose of outpatient EPOCH-FR therapy; for patients whose CD4 count is above 200 cells/µl, the patient is assigned to arm IV and receives the transplant after the maximum of three cycles of EPOCH-FR therapy and then after a Flu/Cy preparative regimen that is reduced by 75% (total cyclophosphamide dose, 1200 mg/m^2). On arm V, Th2 cells are administered on day 0 of transplant in an attempt to prevent rejection with the transplant approach that does not use the Flu/Cy preparative regimen. On arm IV, Th2 cells are administered on day 14 post-transplant to dissociate any Th2 cell effect from any potential toxicity of the preparative regimen and first two weeks of alloengraftment.

1.2.8 Th2.rapa Cell Infusion After Low-intensity Host Preparation ("Arm IV"): Preliminary Results and Rationale for Expansion of Accrual

At this stage in protocol implementation (at the time of amendment N submission), 17 patients have been transplanted on arm IV (low-intensity chemotherapy with Th2 cell infusion at day 14 post-transplant). Current clinical data indicate that transplantation on arm IV is a potentially promising transplant approach. First, this regimen appears to be relatively safe: in 17 patients transplanted on arm IV, there has been no case of transplant-related-mortality. Second, 100% of patients (17/17) have easily achieved the protocol-defined engraftment goal (attainment of >50% donor T cell chimerism by day 100 post-transplant). As early as day 28 post-transplant, 14/17 patients achieved the protocol objective, with a median donor T cell chimerism of 91% (range, 44 to 100%); by day 56 post-transplant, 17/17 patients achieved the engraftment endpoint. And third, this regimen has been associated with a low rate and severity of acute GVHD. Specifically, only 12% of patients (2/17) developed grade II to IV acute GVHD; in each of these two cases, acute GVHD was limited to mild gut GVHD (each case was stage 1 gut, grade II overall GVHD) and responded quickly to corticosteroid therapy.

The statistical rationale for increasing accrual to protocol arm IV is based on the new objective of determining a more precise estimate of the fraction of patients that develop clinically significant acute GVHD (grade II to IV). That is, the 12% rate of acute GVHD that is limited to grade II severity is potentially favorable relative to current results in the field using other regimens. The current protocol, which emphasized engraftment as the statistical objective, limits arm IV accrual to 25 patients. For the new statistical objective of providing a more precise estimate of the acute GVHD, it will be favorable to increase arm IV accrual to a total of 40 subjects. This accrual number was determined after consultation with the protocol statistician,

Dr. Seth Steinberg. If arm IV enrollment is continued until a total of 40 patients have been treated, the maximum confidence interval width for the fraction of patients with grade II to IV acute GVHD will not exceed +/- 15%.

1.3 BACKGROUND AND RATIONALE: SUMMARY

In protocol 99-C-0143, we identified that augmentation of T cell replete allografts with CD28 co-stimulated donor Th2 cells was feasible, could be safely performed at Th2 cell doses of 5 to 125 x 10^6 Th2 cells/kg, and was associated with donor T cell expansion and a mixed type I and type II cytokine profile post-transplant. Using single agent cyclosporine GVHD prophylaxis, the incidence and severity of acute GVHD was not significantly reduced in Th2 recipients, with gut GVHD representing the primary site of GVHD involvement. In recent murine studies, we have found that addition of sirolimus during T cell expansion generates Th2 cells with enhanced capacity to prevent acute GVHD, in particular, gut GVHD. Sirolimus generated Th2 cells can be promoted in vivo through post-transplant administration of short-course sirolimus, with consolidation of Th2 cell modulation of alloreactivity. In further studies, we have modified the clinical Th2 cell culture method to include high dose sirolimus; such sirolimus generated human Th2 cells have an enriched Th2 cytokine phenotype, with reduced expression of the Th1-type molecules IL-2, IFN-γ, and CD40 ligand, and can be easily generated with a single round of expansion in 20 days or less. These results provide the basis for this second generation Th2 cell protocol, which will evaluate the new Th2 cell product at an established dose (2.5 x 10^7 Th2 cells/kg) in the setting of post-transplant immune suppressive therapy with cyclosporine combined with short-course sirolimus therapy. This therapy will be administered after low-intensity host preparation, which will be tailored to each subject based upon the level of host immune depletion, as defined by $CD4^+$ T cell count.

1.4 PROTOCOL PROGRESS TO SUPPORT AMENDMENT P

Protocol 04-C-0055 is currently accruing only to arm IV of the study due to the favorable clinical results observed on this treatment arm. On arm IV, patients with refractory hematologic malignancy receive a low-intensity allogeneic hematopoietic stem cell transplant at a time when the patient is relatively modestly immune-depleted (as defined by a host CD4+ T cell count of < 200). Some patients may express a CD4 count < 200 at the time of study entry and are therefore immediately eligible for transplant; in the event that a patient's CD4 count is > 200 at the time of study entry, EPOCH-F is administered until the CD4 count is < 200, at which time the patient then proceeds to transplantation. Just prior to transplant, arm IV patients receive a preparative regimen containing fludarabine in combination with a dose of cyclophosphamide that is 75% reduced relative to our previously utilized dose (reduction from 4800 to 1200 mg/m2). GVHD prophylaxis on arm IV consists of a short-course of sirolimus through day 14 post-transplant in combination with cyclosporine, which is intended to be administered through day 100 post-transplant and gradually tapered off by day 180 post-transplant. Finally, at day 14 post-transplant, arm IV patients receive a donor lymphocyte infusion that consists of ex vivo generated Th2 cells that are manufactured in sirolimus (Th2.rapa cells) by a 12-day culture method.

At this point in protocol implementation, 28 subjects have received donor Th2.rapa cells on arm IV therapy. This therapy has been relatively safe. First, because of the low-intensity preparative regimen, neutropenia has either been non-existent or of limited duration (typically, < 7 days). Second, there has not been a single case of engraftment syndrome after the allogeneic HSCT

(0/28 cases); of note, severe engraftment syndrome was a dose limiting toxicity of Th2.rapa cell therapy when such cells were administered on day 0 of transplant in the context of the full-dose preparative regimen (previous arm I and arm II therapy). In spite of the relatively low-intensity preparative regimen, alloengraftment has typically occurred relatively early post-transplant. Specifically, the average percent donor T cell chimerism on arm IV recipients has been 59.2%, 81.5%, and 89.1% at days 14, 28, and 100 post-transplant; similarly, the average percent donor myeloid cell chimerism has been 42.2%, 73.9%, and 87.7% at days 14, 28, and 100 post-transplant. The incidence of graft rejection has been 1/28 (3.6%); of note, rejection in this patient was likely precipitated by the necessity to administer chemotherapy in the first month post-transplant to treat rapidly progressive malignancy. And third, the incidence of acute GVHD on arm IV has been remarkably low at 3/28 patients (10.7%); in each of these cases, the acute GVHD has been of moderate severity (grade II or III out of IV) and responsive to steroid therapy. Chronic GVHD, which in known to associate with effective graft-versus-tumor responses, has been observed in 11/17 evaluable patients (64.7%). Overall, 23 out of 28 Th2.rapa cell recipients transplanted on arm IV are alive at a median of 289 days post-transplant (range, day 42 to day 1186 post-transplant). The five post-transplant deaths in Th2.rapa cell recipients on arm IV were each related to progressive malignancy at days 91, 119, 194, 211, and 748 post-transplant. The currently active protocol design (version, amendment O) specifies that 40 patients will be accrued to arm IV, with the primary objective to determine the safety, feasibility, alloengraftment, and GVHD rate on arm IV. Currently, the final 12 patients to meet this n=40 accrual goal are either already enrolled to the study or will be anticipated to enter the study in the next 2-3 months.

The proposed amendment P seeks to accrue twenty additional patients and maintain each of the treatment parameters specified in the current arm IV therapy, with the one exception being the method of manufacture of the donor Th2.rapa cell product. Currently, arm IV patients receive Th2.rapa cells that are manufactured in the NIH Department of Transfusion Medicine (DTM) for a total span of 12-days in culture; in the proposed amendment, we seek to evaluate donor Th2.rapa cells that have been manufactured in a shorter interval, 6-days. This amendment would effectively yield an evaluation of 40 patients on arm IV using 12-day cultured Th2.rapa cells ("Arm IVA") and 20 patients on arm IV using 6-day cultured Th2.rapa cells ("Arm IVB"). The rationale for evaluation of the 6-day method of Th2.rapa cell manufacturing includes: (1) in terms of feasibility, reduction of time in culture will facilitate donor and recipient scheduling; (2) the reduced culture time will significantly reduce the expense of Th2.rapa cell manufacturing because of reduced culture media and recombinant cytokine utilization, reduced need for skilled labor to split and feed cultures, and will reduce the chance of culture contamination; and (3) potentially, reducing the time of Th2.rapa cell manufacturing from 12 to 6 days may improve the in vivo function of the adoptively transferred T cell product. That is, T cells propagated for a shorter interval have undergone a limited number of cell cycle divisions, thereby potentially allowing for greater in vivo replicative potential. Of note, our recently published murine experiments involving donor Th2.rapa cells utilized a 6-day manufacturing method. Importantly, working with the NIH DTM, we have validated in clinical scale cultures that the human Th2.rapa cells manufactured by the 6-day method express a Th2 cytokine phenotype. The IND for Th2.rapa cells (#11832;) is being amended to include the 6-day culture method.

1.5 PROTOCOL PROGRESS TO SUPPORT AMENDMENT R

Arm IVA evaluated low-intensity allogeneic HSCT using a pre-emptive donor lymphocyte infusion (DLI) at day 14 post-transplant; the DLI cell product consisted of Th2 cells that were manufactured by a 12-day culture method. Arm IVA therapy was relatively safe: there were no cases of engraftment syndrome (0/40), a low rate of grade II-IV acute GVHD (4/40 cases; 10%), and no case of transplant-related mortality. Malignant disease progression has been the primary therapeutic limitation of arm IVA therapy. It should be noted that patients accrued to arm IVA each had measurable disease and had progression of disease after multiple prior regimens (median of 3.5 prior regimens); in addition, the majority of patients transplanted on arm IVA had malignancies that are considered high-risk for disease progression or malignancies that were not sensitive to prior chemotherapy. Of the 40 patients transplanted on arm IVA, there have been 15 cases of progressive malignancy that have led either to patient death (n=12), patient removal from study (n=2), or loss of alloengraftment after chemotherapy treatment (n=1). Of the remaining 25 patients, 21 are in complete remission; the remaining four (4) of 25 patients are receiving continued therapy for persistent malignancy post-transplant. Complete remission has been less commonly observed for patients with high-risk disease; that is, remission has been observed in 5/12 (42%) of patients with chemotherapy refractory B cell malignancy, 1 out of 4 (25%) of Hodgkin's Disease patients, and 0 out of 4 (0%) of patients with AML. In total, 6/20 (30%) of such high-risk patients have achieved disease remission. Increased potency of anti-tumor effects is generally observed when a patient achieves full donor lymphoid engraftment post-transplant. For arm IVA, the frequency of patients that have achieved full donor lymphoid engraftment (≥ 95% donor) at day 100 post-transplant was 17/37 cases (46%). Given this information, we reason that the relatively frequent occurrence of mixed donor lymphoid chimerism on arm IVA may represent a barrier to more effective anti-tumor effects post-transplant, particularly in patients with high-risk malignancy.

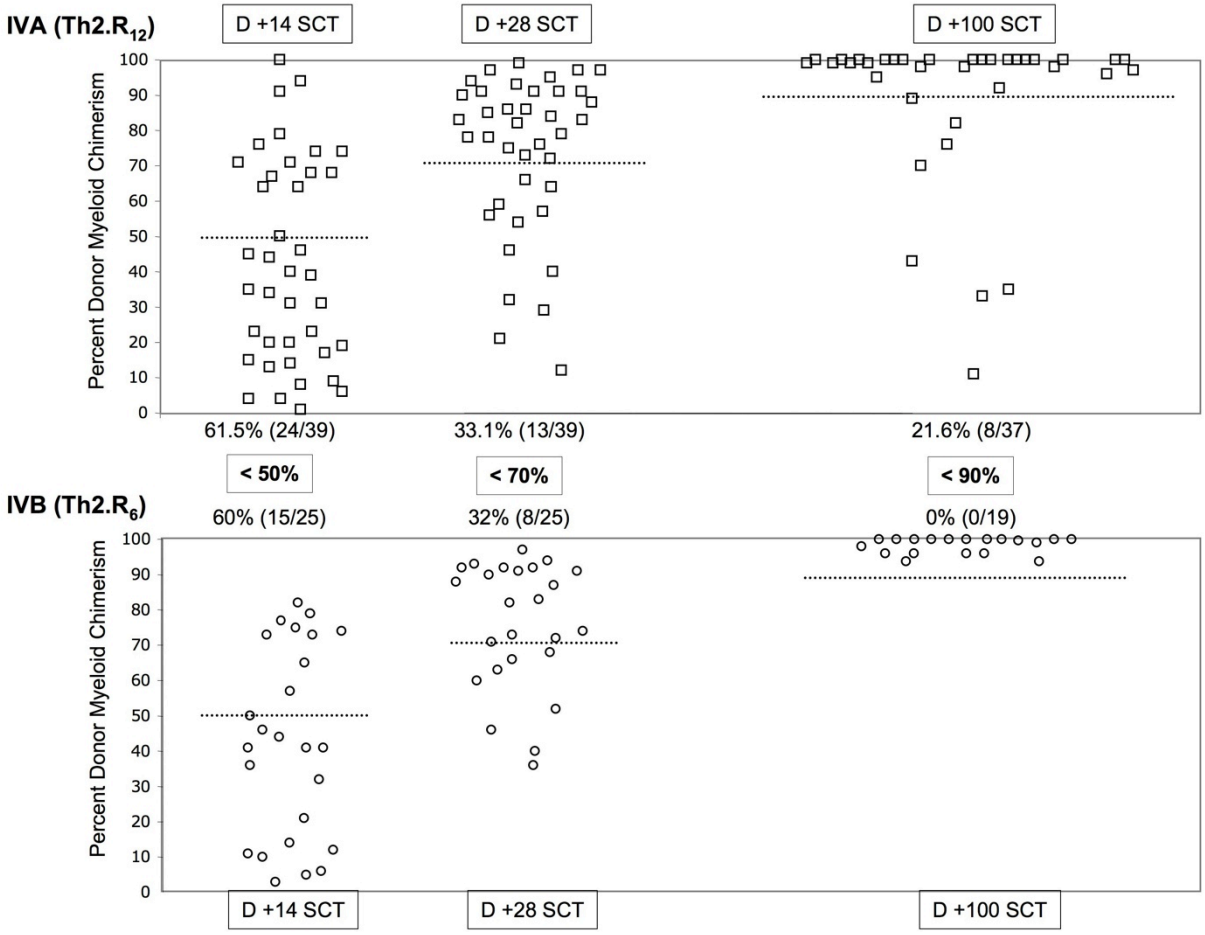
Arm IVB was implemented to evaluate the effect of pre-emptive DLI using donor Th2 cells manufactured by a shorter, 6-day culture method; aside from this one difference (12-day vs. 6-day manufacturing of Th2 cells), there is no difference between arm IVA and arm IVB therapy. Our hypothesis was that the 6-day manufactured Th2 cell would mediate increased in vivo effects, thereby leading to more cases of complete donor lymphoid engraftment and a resultant increase in anti-tumor effects. The initial primary objective of arm IVB was to determine the acute GVHD rate in a cohort of 20 patients. At this point in protocol implementation, 5 out of 14 patients (36%) treated on arm IVB have developed grade II-IV acute GVHD; while this incidence is not increased relative to standard transplant approaches, it is likely not consistent with the very low rate of acute GVHD that we observed on arm IVA (10%). These data indeed support our hypothesis that the 6-day manufactured Th2 cell may mediate more potent in vivo effects than the 12-day manufactured Th2 cell. Importantly, each of the five cases of acute GVHD that have occurred on arm IVB has been responsive to cortico-steroid therapy.

In addition, early data indicates that arm IVB therapy may be associated with a higher frequency of full donor lymphoid engraftment. That is, 4 out of 5 (80%) of fully evaluable recipients on arm IVB have full donor lymphoid engraftment at day 100 post-transplant; by comparison, only 46% or arm IVA patients had full donor lymphoid engraftment at day 100 post-transplant. With amendment R, a new primary protocol objective will be to assess whether the frequency of patients that achieve full donor lymphoid engraftment can be increased from 46% (arm IVA results) to a goal of 80% of arm IVB patients. We also hypothesize that arm IVB therapy will

result in more potent anti-tumor effects. Indeed, promising anti-tumor effects have been observed in the initial patients treated on arm IVB. Specifically, 8 of the initial 10 patients transplanted on arm IVB have attained a partial or complete response; the majority of these patients had high-risk malignancy. With amendment R, we will test this anti-tumor hypothesis in a preliminary manner by addition of the following secondary study objective. Specifically, we seek to increase the frequency of patients with high-risk malignancy who achieve complete remission from 30% (arm IVA) to 70% (arm IVB). In sum, by comparison of n=40 patients each transplanted on arms IVA and IVB, we will be able to compare the 12-day and 6-day Th2 cells as a pre-emptive DLI therapy for their safety (in terms of GVHD) and their efficacy (in terms of promoting engraftment and anti-tumor effects).

1.6 PROTOCOL PROGRESS TO SUPPORT AMENDMENT U

After accrual of n = 25 patients to arm IVB, clinical results indicate that the 6-day manufactured Th2 cell evaluated on arm IVB promote alloengraftment more effectively than the 12-day manufactured Th2 cell evaluated on arm IVA. The figure below shows the myeloid cell chimerism data for arms IVA (Th2.R_{12}) and arm IVB (Th2.R_6) at days 14, 28, and 100 post-transplant. At day 14 post-transplant, just prior to infusion of the 12-day (arm IVA) or 6-day (arm IVB) manufactured Th2 cells, a similar frequency of patients had a level of donor myeloid chimerism of < 50% (61.5% of recipients in arm IVA vs. 60% of recipients in arm IVB).



At day 28, 14 days after pre-emptive Th2 cell DLI, the frequency of patients with a level of donor myeloid cell chimerism of < 70% was 33.1% in arm IVA and 32% in arm IVB. However, by day 100 post-transplant, the frequency of patients with a level of donor myeloid cell chimerism of < 90% was 21.6% in arm IVA and 0% in arm IVB. The conversion of mixed myeloid cell chimerism to full donor myeloid chimerism occurs primarily through an allogeneic, T cell-mediated immune response against residual host myeloid cells, thereby creating space for donor engraftment. These data therefore indicate that the 6-day manufactured Th2 cell evaluated on arm IVB may more effectively eliminate host targets; and, by extension, it is possible that this increased allogeneic immune function in arm IVB recipients might translate into improved graft-versus-tumor effects. A reduced degree of mixed chimerism in the T cell compartment was also observed in arm IVB relative to arm IVA (not shown).

It is difficult to determine whether GVT effects are indeed enhanced on arm IVB relative to arm IVA because of the heterogeneous patient populations, the current difference in number of patients evaluated (although at the end of accrual, 40 patients will be accrued to each arm), and the limited patient follow-up of patients on arm IVB. Nonetheless, it is important to note that the rapid conversion to full donor chimerism observed on arm IVB has been associated with clear GVT effects: 10/25 evaluable patients have so far achieved a complete remission and 4/25 patients are currently in a partial remission. As such, arm IVB therapy yielded rapid and complete alloengraftment and demonstrable GVT effects. Therefore, our further efforts in low-intensity allogeneic transplantation will further evaluate the 6-day manufactured Th2 cell.

Graft-versus-host disease (GVHD) remains the most important limitation of arm IV therapy, and therefore, an attempt to improve GVHD outcome will be the focus of our next effort using 6-day manufactured Th2 cells in low-intensity transplantation. Acute GVHD of grade II to IV severity has been observed in 4/40 patients on arm IVA (10%) and 7/25 patients on arm IVB (28%). It is not clear that this difference in incidence reflects a real difference in the capacity of 12-day vs. 6-day manufactured Th2 cells to mediate acute GVHD or reflects other differences between the cohorts (such as a higher incidence of gastro-intestinal infections as a co-factor for GVHD in arm IVB [C.diff. and CMV infections]). It should also be noted that chronic GVHD has been observed in the majority of patients on both arms IVA and IVB, with incidences of 64.7% (22/34) and 53.3% (8/15), respectively. As such, we have reasoned that our next clinical trial efforts should seek to limit the negative consequences of GVHD associated with arm IV therapy.

It is towards this aim that we propose the current amendment to evaluate arm IV therapy on a new cohort of patients (arm IVC; n = 40 to be accrued) that will receive an intensified GVHD prophylaxis regimen that will target a higher steady state level of sirolimus (new target range, 20 to 30 ng/ml; increased from previous target range of 3 to 12 ng/ml). As shown in the table below, we have found that recipients of GVHD prophylaxis consisting of cyclosporine plus moderate dosing of sirolimus express T cell inflammatory cytokines at days 7 and 14 post-transplant; that is, T cells harvested from transplant recipients on arm IV at these early time points secrete high levels of IL-2, IFN-γ, and TNF-α in spite of the double-agent GVHD prophylaxis.

GVHD Prophylaxis Using Cyclosporine Plus Short-Course Sirolimus (target sirolimus level of 3 to 12 ng/ml) Does Not Control T Cell Inflammatory Cytokines At Days 7 and 14 Prior to Pre-emptive Infusion of Th2 Cell DLI

Cytokine Capture Flow[2]	Prior to T-rapa Infusion		After T-rapa Cell Infusion
	Day +7	Day +14	Day +28
% $CD4^+IFN\text{-}\gamma^+$	21.1 ± 4.7	14.7 ± 2.7	32.5 ± 5.8
% $CD8^+IFN\text{-}\gamma^+$	28.0 ± 4.8	14.8 ± 2.7	35.7 ± 6.0
% $CD4^+IL\text{-}2^+$	14.5 ± 2.2	13.1 ± 3.1	30.7 ± 5.0
% $CD8^+IL\text{-}2^+$	9.9 ± 1.3	10.0 ± 3.2	18.8 ± 3.6
% $CD4^+IL\text{-}4^+$	6.5 ± 0.9	9.8 ± 2.2	15.3 ± 2.9
% $CD8^+IL\text{-}4^+$	6.8 ± 0.9	9.2 ± 2.7	11.9 ± 2.6
% $CD4^+IL\text{-}10^+$	15.1 ± 2.2	19.9 ± 3.7	24.6 ± 3.1
% $CD8^+IL\text{-}10^+$	14.2 ± 1.4	17.5 ± 3.8	20.2 ± 2.5

T Cell Supernatant[3]	Prior to T-rapa Infusion		After T-rapa Cell Infusion	
	Day +7	Day +14	Day +28	Day +50
IFN-γ	957 ± 487	246 ± 114	976 ± 722	1165 ± 550
IL-2	2058 ± 1334	124 ± 29	1601 ± 1093	706 ± 214
TNF-α	7617 ± 6475	310 ± 112	413 ± 204	438 ± 118
IL-17	33 ± 1	< 1	108 ± 108	42 ± 36
IL-4	4 ± 4	2 ± 2	20 ± 15	8 ± 5
IL-5	105 ± 10	2 ± 1	157 ± 103	100 ± 57
IL-10	5 ± 2	7 ± 1	24 ± 12	18 ± 7
IL-13	22 ± 15	14 ± 7	218 ± 145	167 ± 57

[1] Patients received fludarabine (30 mg/m2/d x 4) and low-dose cyclophosphamide (300 mg/m^2/d x 4) prior to G-CSF mobilized, T cell-replete, matched sibling HCT. Ex vivo generated donor T-rapa cells were administered as a pre-emptive DLI at day +14 post-HCT.

[2] Peripheral blood cytokine capture flow cytometry was performed at days +7, +14, and +28 post-HCT. Cell surface lineage markers (CD4, CD8) were used in combination with Miltenyi® cytokine capture reagents; assay was performed after CD3, CD28 co-stimulation. Values shown are mean ± SEM of the percent of all CD4 or CD8 cells that are positive for cytokine secretion. For each measurement, values were available on n = 24 to 28 T-rapa cell recipients.

[3] Post-HCT mononuclear cells were harvested at days +7, +14, +28 (± 2 days) and day +50 (± 4 days) post-HCT; cells were subjected to CD3, CD28 co-stimulation, and 24 h supernatants were tested for cytokine content by Luminex® assay. Values shown are mean ± SEM, in pg/ml. For each measurement, values were available on n = 25 to 27 T-rapa cell recipients.

Because the experimental Th2 cells are not administered until day 14 post-transplant, the source of the T cells accountable for this inflammatory state is the T cells contained in the mobilized stem cell product. These results indicate that the current GVHD prophylaxis regimen does not adequately control allogeneic T cell activation prior to the infusion of the experimental Th2 cell product. We have reasoned that this initial phase of donor T cell activation might be effectively inhibited by increasing the target level of sirolimus (new target, 20 to 30 ng/ml) in the current GVHD prophylaxis regimen, thereby providing more effective control of GVHD.

Patients accrued to arm IVC will receive the 6-day manufactured Th2 cell that we are currently evaluating on arm IVB. Therefore, patients on arm IVB and the proposed arm IVC will receive the exact same therapy except for the intensified GVHD prophylaxis on arm IVC. It should be noted that the higher steady-state levels of sirolimus will only be sustained through day 7 post-transplant; importantly, we have utilized this higher dose sirolimus target on protocol 08-C-0088 through day 60 post-transplant, and have observed that this regimen was not associated with significant adverse events and has been associated with an absence of grade II-IV acute GVHD (0/11 cases). Forty patients will be accrued to the new arm IVC. This design will therefore result in 40 patients each being treated on arm IV with standard-dose sirolimus and 12-day manufactured Th2 cells (arm IVA), standard-dose sirolimus and 6-day manufactured Th2 cells (arm IVB), and higher-dose sirolimus and 6-day manufactured Th2 cells (arm IVC). This design will allow us to characterize an acute and chronic GVHD rate for each transplant approach, and will provide valuable information to help us select an arm for future studies in our attempt to define a regimen that yields the most favorable balance between GVHD and GVT effects.

1.7 PROTOCOL PROGRESS TO SUPPORT AMENDMENT AA (FEBRUARY, 2013)

We have developed a new transplant platform that involves novel low-intensity conditioning (fludarabine with 75% reduced dose of cyclophosphamide), novel double-agent GVHD prophylaxis (sirolimus combined with cyclosporine), and novel use of a pre-emptive donor lymphocyte infusion consisting to ex vivo manufactured donor Th2 cells (or "T-Rapa" cells) administered at day 14 post-transplant. More than 100 patients treated with one of three variations of this transplant strategy are now evaluable, and the results are favorable in terms of rapid conversion of mixed chimerism, a rate and severity of GVHD that is not increased, and sustained complete remissions in a patient population with refractory hematologic malignancy. This type of regimen may be of value to the general transplant community, and as such, it is now important to directly compare the current transplant approach to other transplant regimens. As a step in this direction, using our novel transplant platform, we will perform a preliminary investigation to assess the role of the ex vivo manufactured Th2 cells in the transplant outcome; to accomplish this, we will randomize lymphoma patients to receive either the investigational Th2 cells (arm IVD, cohort 1; n=10 evaluable patients) or a T cell population that is administered as a standard transplant intervention (unmanipulated donor T cells; arm IVD, cohort 2; n=10 evaluable patients). We anticipate that data obtained from this amended protocol will facilitate our effort to design and implement new and more definitive randomized clinical trials, with eventual comparison of our low-intensity approach to standard transplant regimens in defined patient populations.

We have developed this new transplant approach on arm IVA (using T-Rapa$_{12}$ cells, manufactured for 12 days ex vivo; n=40 evaluable), arm IVB (using T-Rapa$_6$ cells, manufactured for 6 days ex vivo; n=44 evaluable), and arm IVC (using T-Rapa$_6$ cells and a higher dose of in vivo sirolimus therapy; n=22 evaluable). In each arm, we have observed that patients are initially

typically in a state of mixed donor/host chimerism in both the T cell and myeloid cell lineages at the time of pre-emptive DLI (day 14 post-transplant); and, after DLI, patients convert towards a higher proportion of donor chimerism, which has been associated with enhanced anti-tumor effects both in our studies and in the general transplant literature. Specifically, in arm IVA, the median level of T cell and myeloid cell chimerism values at day 14 post-transplant were 51% and 31%, respectively; after T-Rapa cell DLI, these values increased to 80% and 76% (at day 28 post-transplant) and to 93% and 99% (at day 100 post-transplant). In arm IVB, mixed chimerism was again observed at day 14 post-transplant, with median levels of donor T cell and myeloid cell chimerism of 46% and 46%, respectively; after T-Rapa cell DLI, these values increased to 83% and 88% (at day 28 post-transplant) and to 97% and 100% (at day 100 post-transplant). And, in arm IVC, mixed chimerism was again observed at day 14 post-transplant, with median levels of donor T cell and myeloid cell chimerism of 60% and 52%, respectively; after T-Rapa cell DLI, these values increased to 83% and 88% (at day 28 post-transplant) and to 93% and 100% (at day 100 post-transplant). In sum, these chimerism data indicate that the T-Rapa cell DLI is associated with the conversion of mixed chimerism after low-intensity chemotherapy conditioning. However, it is not clear whether such mixed chimerism conversion might occur spontaneously (without pre-emptive DLI) or might occur through administration of a standard, unmanipulated DLI; in this protocol, we will evaluate the latter possibility (that is, whether a standard, unmanipulated DLI is associated with rapid conversion to full donor chimerism using the low-intensity transplant platform). We have elected to not pursue the option of comparing T-Rapa cell DLI to no DLI because there may be an increased risk of graft rejection if no DLI is administered on our transplant platform. That is, in prior transplant studies involving non-myeloablative conditioning, day 14 post-transplant T cell chimerism values of < 50% have been associated with an increased risk of graft rejection [Reference: Baron F, Baker JE, Storb R, et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood. Oct 15 2004;104(8):2254-2262]. In our transplant studies, approximately 50% of patients are in this category of being at increased risk for graft rejection based on day 14 post-transplant T cell chimerism values.

Conversion of mixed chimerism after T-Rapa cell DLI has been relatively safe as there has been no transplant-related mortality and the rate and severity of GVHD has not been increased. On arm IVA, classical acute grade II-IV GVHD occurred in 10% of patients whereas classical chronic GVHD occurred in 46% of patients. On arm IVB, classical acute grade II-IV GVHD occurred in 41% of patients whereas classical chronic GVHD occurred in 37% of patients. And, on arm IVC, classical acute grade II-IV GVHD occurred in 50% of patients whereas classical chronic GVHD occurred in 35% of patients. For each of the treatment arms, the great majority of surviving patients are in sustained complete remission. On arm IVA, 21/40 patients (53%) are alive at a median post-transplant follow-up of 1236 days; for arm IVB, 27/44 patients (61%) are alive at a median post-transplant follow-up of 688 days; and for arm IVC, 16/22 (73%) patients are alive at a median post-transplant follow-up of 260 days. Firm comparisons across these three treatment cohorts are not possible due to the differences in follow-up and the diversity of diagnoses treated. However, at this point in protocol implementation, the increased rate of acute GVHD observed in arms IVB and IVC (recipients of T-Rapa$_6$ cells) does not appear to be associated with reduced survival; this observation suggests that the T-Rapa$_6$ cell may be associated with increased anti-tumor effects (because death due to malignant disease progression has been the main cause of post-transplant lethality on this protocol). Given these data that

suggest an improved anti-tumor effect of the T-Rapa$_6$ population, we have elected to continue our evaluation of the T-Rapa$_6$ cell population as the subset to be utilized for the day 14 post-transplant DLI.

In an attempt to maximize an opportunity to compare the effects of the T-Rapa DLI to the standard DLI, we will limit the randomization to patients age ≥ 18 with a lymphoma diagnosis and to patients who are projected to be able to complete the protocol-defined therapy through day 180 post-transplant. For example, patients with bulky or relatively rapid disease progression are more likely to receive additional post-transplant interventions and are therefore not optimal candidates for randomization. In addition, patients with very low host CD4 T cell counts (<50 cells/microliter) at the pre-transplant time point are more likely to attain full donor engraftment rapidly post-transplant and are more likely to develop engraftment syndrome toxicity that precludes DLI administration; therefore, such heavily pre-treated patients are not ideal candidates for the randomized aspect of the trial. For patients who are not eligible for randomization (due to age, a non-lymphoma diagnosis or other factor such as rapid disease progression or severe host T cell depletion at the pre-transplant evaluation time point), transplantation will be possible on arm IVD, cohort 3 (n=10 evaluable patients). This cohort 3 will evaluate whether T-Rapa cell DLI might be safely administered after further reduction of pre-transplant chemotherapy (elimination of the low-dose Flu/Cy preparative regimen); in an attempt to ensure engraftment on cohort 3, such patients will be required to have a CD4 count less than 50 cells/microliter prior to transplantation.

1.8 PROTOCOL PROGRESS TO SUPPORT AMENDMENT CC (NOVEMBER, 2013)

The protocol design prior to this amendment CC consisted of three transplant cohorts: 1) cohort 1, patients are randomized to receive low-intensity Flu/Cy conditioning plus experimental T-Rapa cell DLI on day 14 post-transplant; 2) cohort 2, patients are randomized to receive low-intensity Flu/Cy conditioning plus control, unmanipulated DLI on day 14 post-transplant; and 3) cohort 3, in patients who are deemed to be not suitable for randomization, such patients receive transplant remote from any chemotherapy (without a preparative regimen) and with multiple infusions of T-Rapa cells. Only patients with a lymphoma diagnosis, without severe immune depletion, and without rapidly progressive malignancy were eligible for randomization and subsequent transplantation on cohorts 1 and 2. A total of ten patients were to be transplanted on each of the three cohorts. At this point in protocol implementation and screening of potential subjects (November, 2013), only two patients were suitable for randomization; in contrast, we have enrolled or are screening nine patients suitable for transplantation on cohort 3. Given this situation, we have amended the protocol (version CC) in an attempt to enhance accrual to the randomized arms; and, because of promising initial results in cohort 3 patients, we have amended the protocol to increase accrual to cohort 3 to a total of 40 patients. Specifically, we have added the diagnosis of chronic lymphocytic leukemia to the list of diagnoses that are eligible for the randomized aspect of the study; in addition, we will work with the multi-center site (Hackensack University) to improve their accrual to the protocol.

The rationale to expand cohort 3 is based on initial results using this highly novel regimen, which performs transplant without a preparative regimen; this strategy is not being evaluated elsewhere, in large part due to the high chance of graft rejection in the absence of conditioning. The first six patients treated on cohort 3 have achieved prompt donor engraftment.

The lack of rejection in cohort 3 therapy is likely due to use of: pre-transplant reduction of host immunity (CD4 count < 50); use of high-dose sirolimus in the post-transplant interval; and pre-emptive DLI using donor T-Rapa cells. To date, albeit with limited patient follow-up, no patient on cohort 3 has experienced neutropenia and no patient has developed acute GVHD. Successful transplantation without a preparative regimen has several potential advantages, including a likely reduced risk of infection (due to minimization of neutropenia) and a likely reduced risk of acute GVHD (the intensity of host conditioning is known to potentiate GVHD). In the amended protocol, we have chosen to expand cohort 3 therapy to a total of 40 patients; this is the number of patients transplanted on previous protocol arms, thereby enhancing statistical comparisons across transplant approaches. Specifically, as detailed in the amended protocol statistical section, we now hypothesize that the rate of grade II to IV acute GVHD in cohort 3 will be reduced to a level of 15% (6 cases out of 40) when compared to the previous rate of acute GVHD of 41% that was observed in the most recent protocol arms (35/86 cases). To help ensure patient safety during cohort 3 implementation, stopping rules have been instituted for an excess rate of severe acute GVHD or an excess rate of death due to malignant disease.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria: Patient (Recipient)

a) Patients with hematologic malignancies, myelodysplasia, or myeloproliferative disorders, as summarized in the following table. The diagnosis must be histologically confirmed by the Laboratory of Pathology of NCI or Hackensack (there will be no central pathology review).

b)

Disease	Disease Status
Chronic Lymphocytic Leukemia	a) Relapse post-fludarabine b) Non-CR after salvage regimen
Hodgkin's and Non-Hodgkin's Lymphoma (all types, including Mantle Cell Lymphoma)	a) Primary treatment failure b) Relapse after autologous SCT c) Non-CR after salvage regimen
Special Cases of High-Risk Lymphoma, including but not limited to: (1) plasma dendritic cell type [Ref: Dermatol Online J, 2008; 14(11):5]; (2) Hepato-splenic T cell type [Ref: Annals of Oncology, Feb 23, 2009]; (3) gamma delta pinniculitic T cell type [Ref: Dermatol Clin, 2008; 26(4):529-40]; (4) muco-cutaneous NK cell type; and (5) stage III-IV nasal NK cell type [Ref for (4) and (5): Cancer, 2008; 112(7): 1425-36]	a) Primary treatment failure b) Relapse after autologous SCT c) Non-CR after salvage regimen d) In first CR or any later CR
Chronic EBV-associated lymphoproliferative disease [Ref: Am J Hem, 2008; 83:721-27][Ref: Clin Inf Diseases, 2008; 46:1525-34]	a) At any point after diagnosis, including up-front therapy
Multiple Myeloma [Reference for sequential autologous to allogeneic transplant strategy: NEJM, 2007; 356(11):1110-20]	a) Primary treatment failure b) Relapse after autologous SCT or for consolidation after autologous SCT c) Non-CR after salvage regimen
Acute Myelogenous Leukemia	a) CR #1 and "high-risk" [excludes t(8;21), t(15;17), or inv(16)] b) CR #2 or greater
Acute Lymphocytic Leukemia	a) CR #1 + "high-risk" [t(9;22) or *bcr-abl*$^{+}$; t(4;11), 1(1;19), t(8;14)] b) In CR #2 or greater
Myelodysplastic Syndrome	a) RAEB b) RAEB-T (requires marrow and blood blasts < 10% after induction chemotherapy)
Myeloproliferative disorders*	a) Idiopathic myelofibrosis b) Polycythemia vera c) Essential thrombocytosis d) Chronic myelomonocytic leukemia
Chronic Myelogenous Leukemia	a) Chronic phase CML, refractory to imatinib treatment b) Accelerated phase CML

- Patients with myeloproliferative disorders must be end-stage, which is primarily defined as disease severity refractory to splenectomy [further defined in ref.(59)].

c) Patient age of 16 to 75 years.
d) Consenting first degree relative matched at 6/6 HLA antigens (A, B, and DR).
e) Patient or legal guardian must be able to give informed consent.
f) All previous intravenous therapy administered outside of the NIH Clinical Center must be completed at least 2 weeks prior to study entry, with recovery to ≤ non-hematologic grade 2 toxicity of previous therapy.
g) ECOG performance status equal to 0 or 1.
h) Life expectancy of at least 3 months.
i) Patients with acute leukemia must have chemotherapy sensitive disease, as defined by at least a 50% reduction in circulating absolute blast count due to the most proximal regimen.
j) Left ventricular ejection fraction ≥ 45%, preferably by 2-D echo, or by MUGA. However, patients with LVEF of between 35% and 44% may also be eligible provided that such

patients are cleared by a Cardiology Consultation; in such cases, Cardiology will determine whether it is appropriate to perform a cardiac stress test.

k) Corrected DLCO > 45% of expected value.
l) Creatinine ≤ 1.5 mg/dl or creatinine clearance ≥ 50 ml/min.
m) Serum total bilirubin < 2.5 mg/dl; serum ALT and AST ≤ 2.5 times upper limit of normal. Values above these levels may be accepted, at the discretion of the PI or LAI, if such elevations are thought to be due to liver involvement by malignancy or GVHD.
n) Adequate central venous access potential.
o) Potential patients referred for the study may not be eligible for the experimental protocol therapy due to reasons such as uncertainty about donor HLA typing or need to control malignant disease, infection, or metabolic abnormality such as hypercalcemia on an emergent basis. Should a referred patient present to us in such a scenario, the patient will be referred back to their primary hematologist-oncologist for treatment. However, if referral back to the referring physician is not in the best interest of the patient according to the clinical judgment of the PI, then the patient may receive standard treatment for the malignant disease or complicating conditions (infection, metabolic problems) under the current study. In other cases, a patient may have reasonable control of malignancy but does not meet the CD4 cell cut-off of 100 cells per microliter required for cohort 3 therapy (or, ALC value of < 300); in such cases, standard care chemotherapy regimens may be administered for the specific goal of reducing the CD4 count (that is, immune depleting regimens such as the pentostatin plus cyclophosphamide combination, administered similar to the manner that we have developed on protocol 08-C-0088). If it becomes apparent that the patient will not be able to proceed to experimental therapy, then he/she must come off study. Recipient-Subjects receiving a standard therapy will be told about the therapy, associated risks, benefits alternatives of the proposed therapy, and availability of receiving the same treatment elsewhere, outside of a research protocol. Because such standard care therapy is not experimental, it is not necessary to complete the eligibility criteria prior to receiving such standard care; however, prior to initiation of the experimental therapy, the patient must meet each of the eligibility criteria detailed above in section **2.1** (subsections a through m). Attempts will be made to standardize such pre-transplant chemotherapy (by administration of EPOCH-FR chemotherapy, which is detailed later in this protocol); however, other regimens using approved agents will be allowed if such regimens are thought to be in the best interest of the patient.

2.1.2 Inclusion Criteria: Donor

a) First-degree relative with genotypic identity at 6/6 HLA loci (HLA- A, B, and DR).
b) Age 11 to 90 years and able to give consent or assent. For donors < 18 years old, the legal guardian must be able to provide informed consent.
c) Adequate venous access for peripheral apheresis, or consent to use a temporary central venous catheter for apheresis.
d) Donors must be HIV negative.
e) Donors with a history of hepatitis B or hepatitis C infection may be eligible. However, eligibility determination of such patients will require a hepatology consultation. The risk/benefit of the transplant and the possibility of transmitting hepatitis will be discussed with the patient and eligibility will then be determined by the principal investigator and LAI.
f) Lactating donors must substitute formula feeding for her infant during period of filgrastim administration (to prevent any filgrastim effect on infant).

2.1.3 Exclusion Criteria: Patient

a) Active infection that is not responding to antimicrobial therapy.
b) Active CNS involvement by malignancy.
c) HIV infection (treatment may result in progression of HIV and other viral infections).
d) Chronic active hepatitis B. Patient may be hepatitis B core antibody positive. For patients with concomitant positive hepatitis B surface antigen, patient will require a hepatology consultation. The risk/benefit profile of transplant and hepatitis B will be discussed with the patient and eligibility determined by the principal investigator and LAI.
e) Hepatitis C infection. Patient may have hepatitis C infection. However, each patient will require a hepatology consultation. The risk/benefit profile of transplant and hepatitis C will be discussed with the patient and eligibility determined by the principal investigator and LAI.
f) Pregnant or lactating. Patients of childbearing potential must use an effective method of contraception. The effects of the chemotherapy, the subsequent transplant and the medications used after the transplant are highly likely to be harmful to a fetus. The effects upon breast milk are also unknown and may be harmful to the infant.
g) History of psychiatric disorder which may compromise compliance with transplant protocol, or which does not allow for appropriate informed consent.

2.1.4 Exclusion Criteria: Donor

a) History of psychiatric disorder which may compromise compliance with transplant protocol, or which does not allow for appropriate informed consent.
b) History of hypertension that is not controlled by medication, stroke, autoimmune disease, or severe heart disease (donors with symptomatic angina will be excluded). Donors with a history of coronary artery bypass grafting or angioplasty who are symptom free will receive a cardiology evaluation and be considered on a case-by-case basis.
c) History of prior malignancy. However, cancer survivors who have undergone potentially curative therapy may be considered for stem cell donation on a case-by-case basis. In addition, donors with localized cancer such as prostate cancer that are on a watch-and-wait management due to the low-risk of disease progression may also be considered for stem cell donation on a case-by-case basis. The risk/benefit of the transplant and the possibility of transmitting viable tumor cells at the time of transplantation will be discussed with the patient.
d) Donors must not be pregnant (unknown effect of filgrastim on fetus). Donors of childbearing potential must use an effective method of contraception.
e) Anemia (Hb < 11 gm/dl) or thrombocytopenia (platelets < 100,000 per μl). However, potential donors with Hb levels < 11 gm/dl that is due to iron deficiency will be eligible as long as the donor is initiated on iron replacement therapy and the case is individually approved by NIH or Hackensack Blood Bank.

2.2 RESEARCH ELIGIBILITY EVALUATION

2.2.1 Recipient (perform within 28 days before study entry unless a different time frame is specified)

a) Complete medical history and physical examination.
b) CT scans of chest, abdomen, and pelvis (include neck CT for lymphoma patients). Patients with CML, AML, ALL, or MDS will not be required to have CT scans.

c) CT or MRI of the head.
d) Cardiac 2-D Echo or MUGA exam
e) For patients who have previously received > 400 mg/m^2 of doxorubicin Chemotherapy, Cardiology Consultation will be obtained; for patients with LVEF between 35% and 44%, both Cardiology Consult and stress test are required.
f) Pulmonary Function Test, including DLCO measurement
g) Skeletal survey, SPEP, and UPEP (only for multiple myeloma patients).
h) Lumbar puncture (as clinically indicated, as determined by PI or LAI).
i) All biopsy specimens will be reviewed by Laboratory of Pathology at either NCI or Hackensack to confirm histologic diagnosis prior to initiation of therapy.
j) CD20 expression by immunohistochemistry or flow cytometry by an accredited referral lab or NCI or Hackensack Lab of Pathology (B cell malignancy patients).
k) Flow cytometric analysis of peripheral blood for CD3, CD4, CD8, and CD56.
l) Antibody screen for hepatitis A, B, and C; HIV, T. Cruzi (Chagas agent), HTLV- I/II, CMV, adenovirus, EBV, HSV, Toxoplasma, and syphilis.
m) PPD test (in recipients considered to be in a high-risk group).
n) CBC with differential, PT, and PTT, and ABO typing.
o) Acute care panel, hepatic panel, and mineral panel. These tests must also be repeated within 72 hours of starting induction chemotherapy.
p) Urinalysis and 24 hour urine collection (for determination of creatinine clearance)
q) Urine βHCG in women of childbearing potential.

2.2.2 Recipient (perform within 90 days before study entry)

a) Social work consultation
b) Chest radiograph
c) Electrocardiogram
d) PET scan (all non-Hodgkin's lymphoma and Hodgkin's lymphoma patients)
e) Unilateral bone marrow aspirate and biopsy (performed only as clinically indicated, as determined by PI or LAI).

2.2.3 Recipient (perform within 6 months before study entry)

a) Dental consultation to assess need for teeth cleaning or removal (within 6 months).

2.2.4 Recipient (perform any time before study entry)

a) Typing for HLA-A, -B, and –DR.
b) PCR test of DNA mini-satellite regions for future determination of chimerism.

2.2.5 Donor (perform any time before study entry)

a) Typing for HLA-A, -B, and –DR.
b) PCR test of DNA mini-satellite regions for future determination of chimerism.

2.2.6 Donor Research Eligibility (perform within 28 days before study entry unless otherwise stated)

a) Complete medical history and physical examination (within 90 days before study entry).
b) Antibody screen for hepatitis A, B, and C; HIV, T. Cruzi (Chagas agent), HTLV-
c) I/II, CMV, adenovirus, EBV, HSV, Toxoplasma, and syphilis (these tests will be repeated within 28 days of stem cell harvest).

d) CBC with differential, PT, and PTT, and ABO typing (these tests will be repeated within 28 days of stem cell harvest).
e) Acute care panel, hepatic panel, and mineral panel (these tests will be repeated within 28 days of stem cell harvest).
f) Urinalysis.
g) Urine βHCG in women of childbearing potential.
h) Chest radiograph (within 90 days).
i) Electrocardiogram (within 90 days).

2.3 PATIENT REGISTRATION AND RANDOMIZATION

a) Protocol "Entry date" is the day that consent forms have been signed by both donor and recipient. "Treatment start date" is the day recipient begins induction therapy.
b) For registration at NCI, authorized staff must register an eligible candidate with Central Registration no later than 24 hours after both donor and patient (recipient) have signed consent forms. To alleviate the burden of additional travel, donors may sign the donor consent form on a provisional basis while awaiting final determination of donor and patient eligibility; thus, the date of donor consent may precede the patient's date of consent by up to four weeks. However, the patient may not sign consent before both the donor and patient have been determined to be eligible, and neither the donor nor the patient will be registered before both have signed consent. Both the patient and the donor must be registered. A registration checklist from the Web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. For questions regarding registration, authorized staff should call Central Registration at 301-402-1732 between the hours of 8:30 a.m. and 5:00 p.m., Monday through Friday; voicemail is available during non-working hours. After confirmation of eligibility, Central Registration staff will call Pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any protocol pharmacotherapy. An assigned patient will not be eligible for the primary GVHD endpoint if that patient does not ultimately receive transplantation (for example, due to chemotherapy toxicity, uncontrolled infection or malignancy, or voluntary withdrawal) or if that patient dies within the first 100 days post-transplant without developing grade II-IV GVHD.
c) For subject registration at Hackensack: Registration will be a two part process as patients are screened on this protocol. A protocol registration form will be supplied by the CCR study coordinator and updates will be provided as needed. Subject eligibility and demographic information is required for registration. To initially register a subject, after the participant has signed consent, complete the top portion of the form and send to CCR study coordinator. Once eligibility is confirmed, after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the patient is being registered for treatment and send to CCR study coordinator. In addition, source documents supporting the eligibility criteria must be sent to the CCR study coordinator. The CCR study coordinator will notify you either by e-mail or fax that the protocol registration form has been received which will include the unique patient/subject ID number. Questions about eligibility should be directed to the CCR study coordinator or PI. Questions related to registration should be directed to the CCR study coordinator.

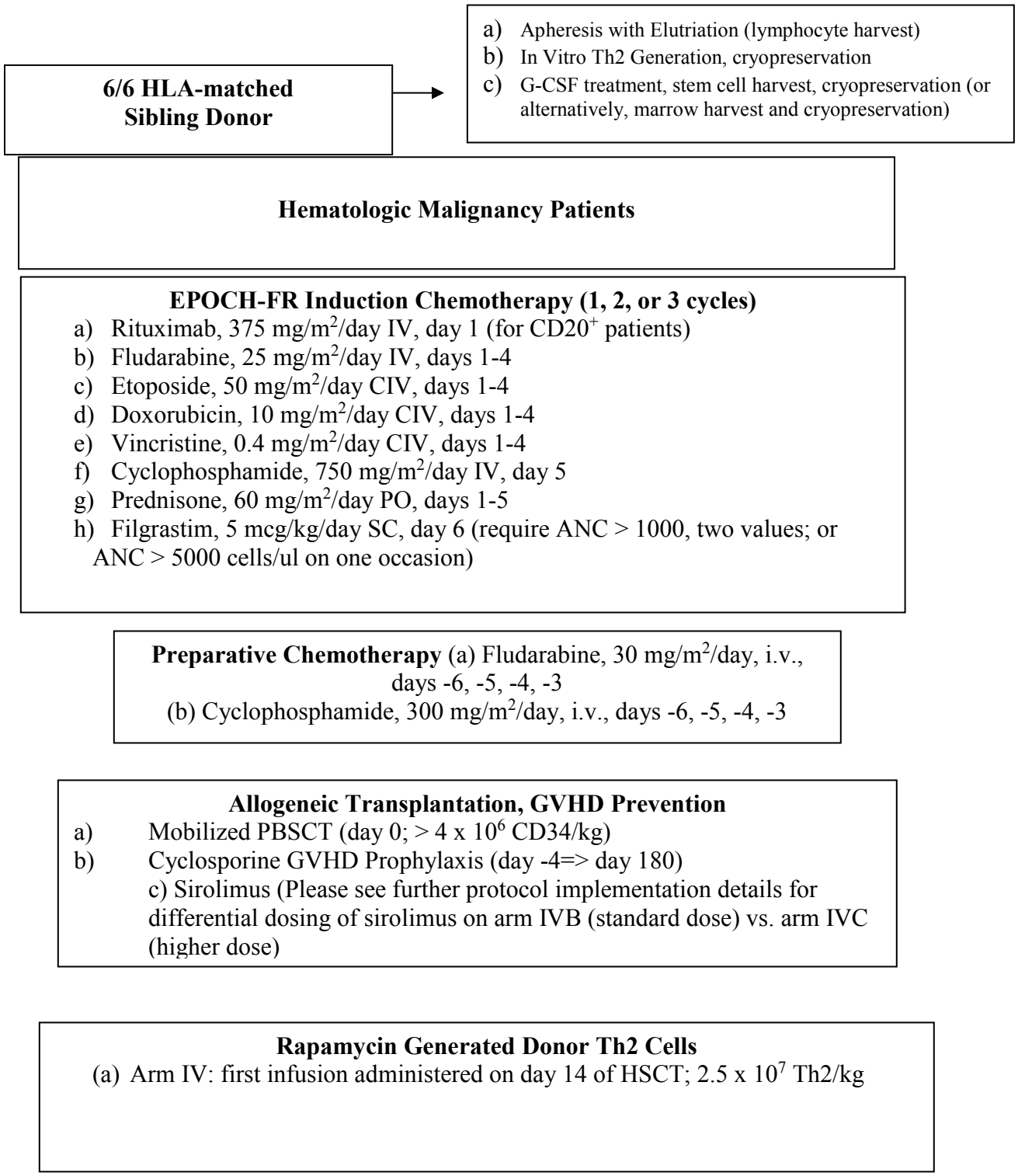
Subjects that do not meet screening criteria should be removed from the study following the procedure in section **3.17.2**.

d) After confirmation of eligibility at Central Registration, Central Registration staff will call Pharmacy at Hackensack to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents.

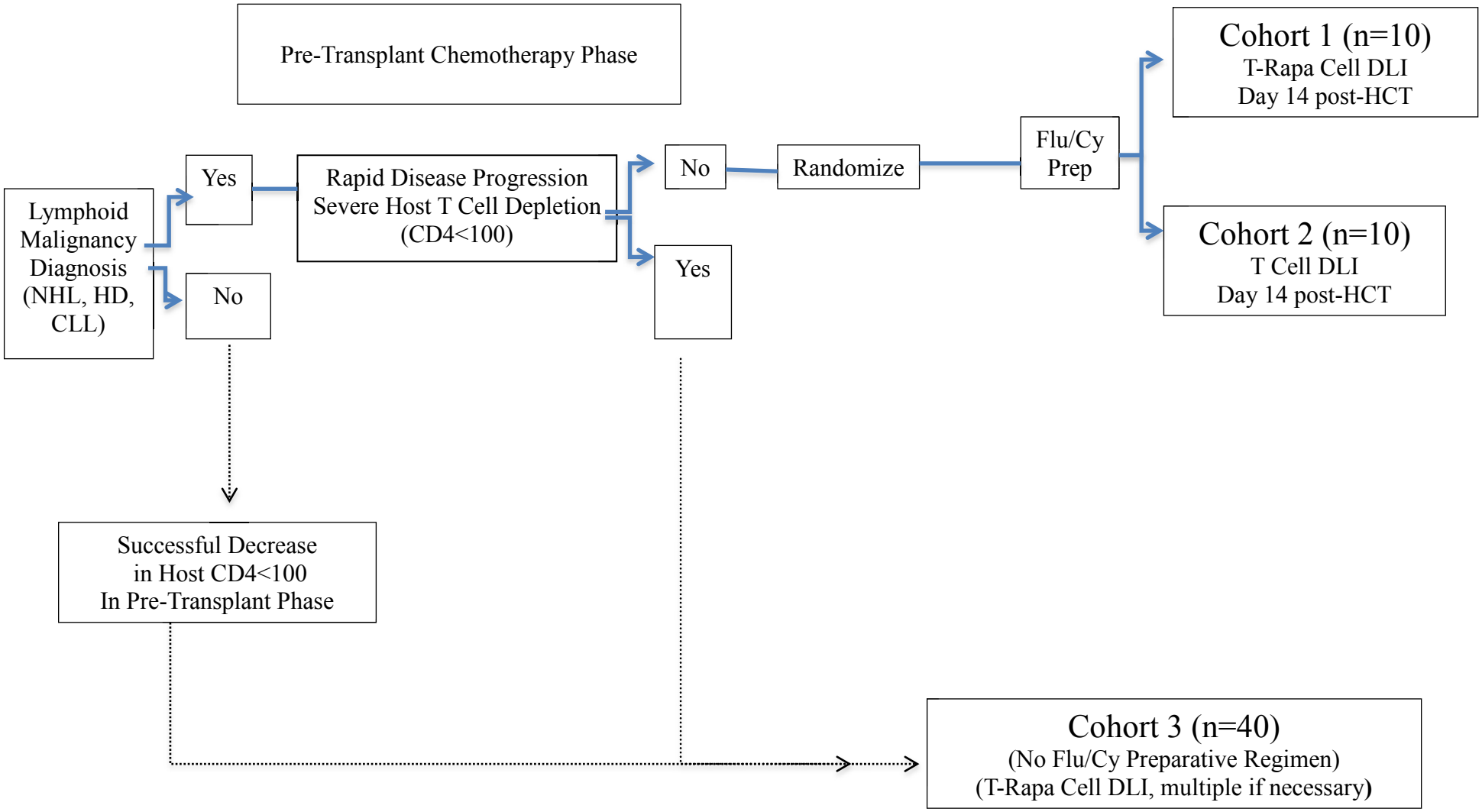
e) Only lymphoma patients 18 years of age and older will be randomized. Block randomization will be made through the Harris contractor under the supervision of Dr. Seth Steinberg. Randomization will occur just prior to administration of the Flu/Cy preparative regimen. Randomization will be stratified according to patients deemed to be at "high-risk" vs. "standard-risk". Patients with chemotherapy refractory lymphoma (stable disease or progressive disease in response to the prior treatment regimen) or patients with Kahl Category III diagnoses (such as diffuse large cell and Hodgkin's Disease) will be considered high-risk (Reference: Kahl et al, BLOOD, 2007 [Oct. 1]); patients with chemotherapy sensitive lymphoma (CR or PR in response to prior therapy) who have Kahl Category I and II diagnoses will be considered as standard risk for the purpose of randomization.

3 STUDY IMPLEMENTATION

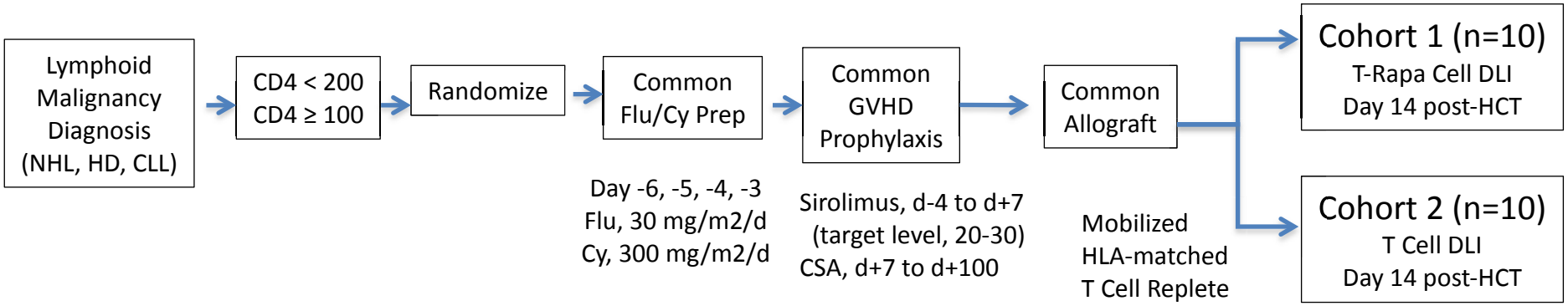
3.1 OVERALL STUDY DESIGN



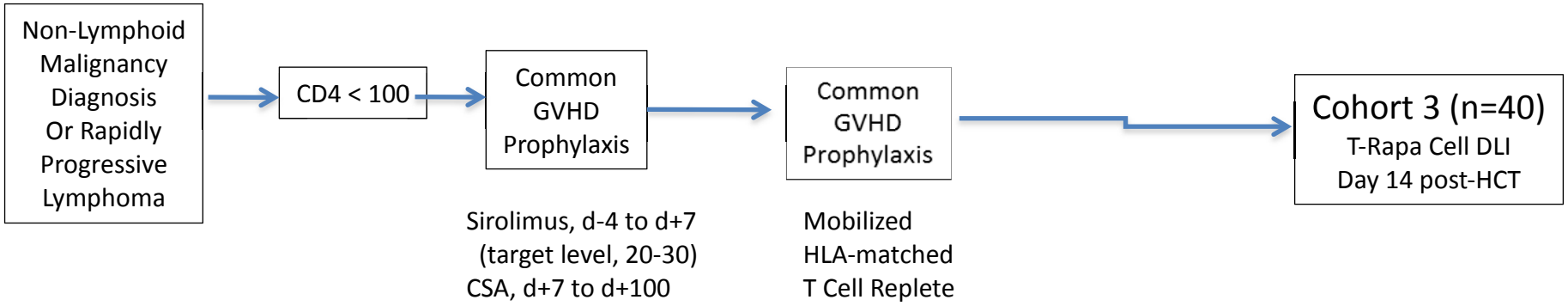
Overall Schema for Protocol 04-C-0055, Arm IVD, Protocol Version CC



Schema for Protocol 04-C-0055, Arm IVD Randomized Cohorts 1 and 2



Schema for Protocol 04-C-0055, Arm IVD Non-Randomized Cohort 3



3.2 STUDY IMPLEMENTATION (DRUG ADMINISTRATION AND TREATMENT MODIFICATIONS)

3.2.1 Lymphocyte Harvest and T Cell Isolation from Donor

a) Donors for patients on the study will undergo a 5 to 15 liter apheresis procedure (CS-3000 or an equivalent machine). The experimental Th2 cells will be generated centrally in the NIH DTM. To accomplish this, donors for recipients treated at Hackensack will undergo the lymphocyte harvest at the NIH DTM.
b) The lymphocyte fraction of the apheresis product will be enriched for $CD4^+$ T cells by CliniMACS® CD4 enrichment. Flow cytometry will be performed to ensure that $CD8^+$ T cells are < 1% of product.
c) The resultant CD4-enriched donor lymphocyte product will be cryopreserved using an NIH DTM protocol in aliquots of 50 to 200 x 10^6 cells/vial. Cell sterility will not be tested at this time (will be tested at end of Th2 cell expansion).
d) For patients under the age of 18, only one apheresis will be allowed (for collection of stem cells). As such, the lymphocyte harvest and T cell isolation in these patients will be performed off of the stem cell product.

3.2.2 Hematopoietic Stem Cell Harvest from Donor

a) Following lymphocyte harvest, donor will receive filgrastim as an outpatient (10 ug/kg/day each morning; subcutaneously) for 5, 6, or 7 days. Donors for recipients to be treated at Hackensack will undergo stem cell mobilization and harvest at Hackensack by the same methodology. In cases where it is anticipated that poor mobilization may occur (increased donor age, Caucasian race, low donor weight, high recipient weight), donors may receive filgrastim at an increased dose of 8 ug/kg BID. Donor should take filgrastim upon awakening in the morning. This is especially important on days 5, 6, and 7 of the injections. A maximum daily dose of 1200ug of filgrastim will be administered.
b) Apheresis will typically be performed on days 5 and 6 of this regimen. On some occasions, sufficient numbers of $CD34^+$ cells might be obtained with a single apheresis on day 5; on other occasions, it may be necessary to perform additional apheresis procedures on days 6 and 7 to reach the target $CD34^+$ cell number ($\geq$ 4 x 10^6 per kg). The donor will be instructed to take filgrastim for the complete 7 day period, unless notified that adequate $CD34^+$ cells were harvested before day 7.
c) If $\geq$ 3 x 10^6 $CD34^+$ cells per kg are harvested after apheresis on days 5, 6, and 7, no further mobilization or apheresis will be performed, and the patient will be eligible to receive the stem cell transplant with that dose of $CD34^+$ cells.
d) In the event that $\geq$ 4.5 x 10^6 $CD34^+$ cells per kg is obtained, the volume of the apheresis product that equals 0.5 x 10^6 $CD34^+$ cells per kg will be set aside for research purposes (specifically, to isolate donor APC populations for immune studies). This sample was picked up by Dr. Fowler's lab, 4-4536.

e) In the event that < 3 x 10^6 $CD34^+$ cells per kg are harvested, donor will be given two weeks of rest, and then will be re-treated with filgrastim (8 ug/kg subcutaneously, BID, for five days) followed by repeat blood stem cell harvesting.
f) A 15 to 25 liter large volume whole blood pheresis will be performed in the NIH DTM via a 2-armed approach or a temporary central venous catheter in the femoral position using the Baxter CS3000Plus, Cobe Spectra, or an equivalent instrument (typically, 4 to 6 hour procedure).
g) Apheresis procedure will use ACD-A anti-coagulant, or heparin.
h) The apheresis product will be cryopreserved and stored at –180° C in Plasmalyte A, Pentastarch, human serum albumin, DMSO, and preservative free heparin (10 U/ml) by the NIH DTM procedure (as defined in BB-IND#9164).
i) The concentration of $CD34^+$ cells in the apheresis product will be determined by flow cytometry, and the number of $CD34^+$ cells in each cryopreserved bag calculated.
j) If donor and host are ABO incompatible, red blood cells will be depleted from the stem cell product by standard DTM protocols.
k) Peripheral blood stem cells should be administered in the range of 4 to 8 x 10^6 CD34 cells per kg recipient body weight.

3.2.3 In Vitro Generation of Donor $CD4^+$ Th2 Cells And Collection of Standard DLI

a) Cryopreserved donor $CD4^+$ T cells will be resuspended to a concentration of 0.3 x 10^6 cells/ml in X-Vivo 20 media supplemented with 5% heat-inactivated autologous plasma.
b) Donor $CD4^+$ T cells will be stimulated with anti-CD3/anti-CD28 coated magnetic beads (3:1 bead:T cell ratio) in filtered flasks at 37° C in 5% CO_2 humidified incubators.
c) At culture initiation, media will be supplemented with sirolimus (1 uM final concentration).
d) At culture initiation and on day 2 of culture, recombinant human IL-4 and recombinant human IL-2 will be added.
e) The median cell volume and concentration of expanded Th2 cells will be determined using a Multisizer II instrument (Coulter).
f) There will be no anti-CD3, anti-CD28 restimulation of expanded Th2 cells.
g) The Th2.rapa cells will be maintained in culture for either 12-days (+/- 1 day) for patients on Arm IVA and for 6-days (+/- 1 day) for patients on Arm IVB. The specific aspect to the Th2.rapa cell manufacturing will be performed in accordance with the ongoing IND, #11832.
h) The following will be the minimal phenotypic requirements of any particular Th2 cell culture to qualify for cryopreservation with subsequent administration. For Th2 cell products to be infused at Hackensack, the cryopreserved product will be shipped by an accredited courier to Hackensack with a certificate of analysis identifying the cell product and attainment of the specific release criteria. Cells not meeting these requirements will be discarded.
    1. Flow cytometry: > 70% $CD4^+$ T cells, and < 5% contaminating $CD8^+$ T cells.
    2. Absence of bacterial and fungal growth.
    3. Absence of endotoxin content by the limulus assay.
    4. Negative mycoplasma test.
    5. After magnetic bead removal, < 100 beads per 3 x 10^6 cells.

6. The target dose for Th2 cells will be 2.5 x 10^7 cells/kg (in unusual cases where T cell manufacturing is limited: minimum allowed dose, 1 x 10^7 cells/kg).

i) The functional characteristics of the expanded Th2 cells will be measured and retrospectively correlated to any observed post-transplant effect, such as toxicity or GVHD outcome. However, in this pilot study, this information will not be utilized as formal release criteria for the cell product. Functional studies will include constitutive and co-stimulated cytokine secretion, with evaluation of type II cytokines IL-4, IL-5, IL-10, and IL-13 and type I cytokines IL-2 and IFN-γ, and flow cytometry to evaluate surface CD25, CD28, CD62L, and CD40L expression.

j) Th2 cell manufacturing will be performed with the goal of generating three separate aliquots to allow for up to three Th2 cell infusions post-transplant (one at the planned day 14 post-transplant time point; two other potential DLI as clinically indicated to treat either persistent mixed chimerism of progressive disease). Th2 cell number in the aliquots will ideally be at a dose of 2.5 x 10^7 cells/kg (minimum dose, 1 x 10^7 cells/kg).

k) For patients to be treated on arm IVD, cohort 2, patients will receive an unmanipulated DLI at day 14 post-transplant. This DLI product will be obtained by steady-state apheresis (will not be G-CSF mobilized) and administered at a dose of 2.5 x 10^7 cells/kg. All DLI products will be cryopreserved.

3.2.4 Tumor Acquisition Prior to Patient Therapy (Optional)

a) An important research objective is to determine whether the curative aspect of transplantation involves an immune component that is specifically directed towards tumor cells. Identification of tumor reactive T cells generated in the allogeneic context may help in the development of new therapies to improve anti-tumor potency.

b) To facilitate this evaluation of tumor reactive T cells, patients will be asked to undergo an optional core biopsy procedure to obtain tumor tissue prior to chemotherapy administration. The Interventional Radiology Department of NIH Clinical Center will evaluate each case for their capacity to obtain tumor tissue; only cases that they deem at low risk for complication will be asked to undergo the core biopsy procedure.

c) Dr. Fowler's lab (4-4536) will pick-up the biopsy specimen from Interventional Radiology, and utilize this material in subsequent in vitro T cell assays.

d) The core biopsy procedure will be primarily applicable to patients with lymphoma. Patients may be asked to undergo bone marrow aspiration or a one-to-two liter single line apheresis procedure for tumor acquisition.

3.2.5 EPOCH-F/R Induction Chemotherapy

a) Adequate central venous access is required for all patients on this protocol.

b) EPOCH-Fludarabine/Rituximab (EPOCH-F/R) outpatient induction chemotherapy will be administered as follows:

EPOCH-F/R Induction Chemotherapy Regimen

Drug	Dose	Days
Rituximab	375 mg/m^2 IV infusion via specified rate titration. (patients with CD20$^+$ malignancies only)	Day 1 only
Fludarabine	25 mg/m^2/day IV infusion over 30 minutes	Days 1, 2, 3, 4
Etoposide	50 mg/m^2/day continuous IV infusion over 24 hrs	Days 1, 2, 3, 4
Doxorubicin	10 mg/m^2/day continuous IV infusion over 24 hrs	Days 1, 2, 3, 4
Vincristine	0.4 mg/m^2/day continuous IV infusion over 24 hrs	Days 1, 2, 3, 4
Cyclophosphamide	750 mg/m^2 IV Infusion over 30 minutes	Day 5 only
Prednisone	60 mg/m^2/day; orally	Days 1, 2, 3, 4, 5
Filgrastim	5 μg/kg/day; subcutaneously	Daily from day 6 (until ANC > 1000/μl x 2 time points; or ANC > 5000/μl x 1)

c) Patients with CD20$^+$ B-cell malignancy will receive rituximab. Pre-medication with acetaminophen 650 mg PO and diphenhydramine 50 mg IV will be administered 30 to 60 minutes prior to each rituximab infusion. Rituximab should be infused prior to the administration of fludarabine and infusional EPOCH chemotherapy.
d) Rituximab infusion should begin at a rate of 50 mg/hr; if no hypersensitivity reaction is observed after 30 minutes, the infusion rate may be escalated in 50 mg/hr increments every 30 minutes, to a maximum rate of 400 mg/hr. If a hypersensitivity reaction occurs, the infusion should be temporarily slowed or halted, with resumption at half the previous rate upon improvement in patient symptoms. If the first infusion is tolerated well, infusions during subsequent cycles of EPOCH-F/R can begin at 100 mg/hr and be increased by 100 mg/hr increments at 30-minute intervals. However, if the first infusion is not tolerated well, then the guidelines for the initial infusion should be followed.
e) Fludarabine will be given immediately after completion of rituximab infusion. If venous access permits, fludarabine should be given concurrently with the start of infusional EPOCH chemotherapy. If venous access does not permit this, fludarabine will be given before the start of EPOCH infusional agents.
f) Total vincristine dose/cycle will not be capped (may exceed 2.0 mg over four days).
g) Cyclophosphamide will be administered on day 5 of each cycle immediately after completion of infusional chemotherapy. One hour prior to cyclophosphamide administration, 1 liter of 0.9% sodium chloride should be infused IV over two hours.
h) For infusional chemotherapy, etoposide, doxorubicin, and vincristine will be combined in a single ('3-in-1') admixture, diluted in a volume of 0.9% Sodium Chloride (NS), Injection, USP, that is based on the amount of etoposide needed to complete one day of treatment:

Etoposide Dose	Volume of Fluid Containing a Daily Dose	Volume of Overfill (fluid + drug)	Volume To Infuse (over 24 hours)	Administration Rate
≤130 mg	528 mL	25 mL	528 mL	22 mL/hour
≥131 mg	1056 mL	25 mL	1056 mL	44 mL/hour

All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine *PLUS* 25 mL overfill (excess) fluid and a proportional amount of drug to compensate for volume lost in administration set tubing.
Before dispensing 3-in-1 admixtures, Pharmacy staff will [1] purge all air from the drug product container, [2] attach an administration set appropriate for use with a portable pump and the set will be [3] primed close to its distal tip and [4] capped with a Luer-locking cap. Bags will be exchanged daily for 4 consecutive days to complete a 96-hour drug infusion. Portable pumps used to administer etoposide + doxorubicin + vincristine admixtures will be programmed to deliver one of two fixed volumes at one of two corresponding fixed rates based on the amount of etoposide and fluid that is ordered (see table, above). At the end of an infusion, some residual fluid is expected because overfill fluid and drug were added; however, nurses are asked to return to the Pharmacy for measurement any drug containers that appear to contain a greater amount of residual drug than expected.

i) Steroids will not be used as an anti-emetic during EPOCH-F/R chemotherapy.
j) Only biweekly ANC values (typically, Monday and Thursday) will determine duration of filgrastim administration, which will be initiated on day 6 at a dose of 5 μg/kg/day. Filgrastim will be discontinued when the ANC is > 1000 cells/μl on two consecutive measurements following the ANC nadir (or > 5000 cells/μl on 1 measurement).

3.2.5.1 Determination of Number of Cycles of Induction Chemotherapy

a) Patients will receive one, two, or three cycles of induction chemotherapy, as determined by the quantity of host immune $CD4^+$ T cells remaining after each cycle.
b) If the CD4 count goes under 200 after cycle 1 or 2 of EPOCH-F chemotherapy, the low intensity preparative regimen will be administered and the transplant will be performed.
c) If the maximum of three cycles of EPOCH-F chemotherapy have been administered and the subject has not yet met the CD4 target (< 200), then the subject will proceed to transplantation on arm IV after the low-intensity fludarabine and cyclophosphamide preparative regimen.
d) A total of 40 patients will be transplanted on arm IVA and 20 patients on arm IVB.
e) At the time of patient entry onto the study, approximately four weeks of time will elapse until the donor Th2 cells and hematopoietic stem cell product will be available for transplantation. For some patients with advanced malignancy, it may not be in the patient's best interest to delay all active therapy during this four-week window. As such, in the event that such a subject is scheduled to receive transplantation after only one cycle of induction chemotherapy (as determined by CD4 cell count), a provision will be made to administer one additional cycle of induction chemotherapy at the time of study entry.
f) The $CD4^+$ T cell count will be measured by a CLIA-certified laboratory at least 48 hours after the cycle's last dose of filgrastim and within three days before the next scheduled cycle (i.e. day 22).

g) Patients will receive the second cycle of chemotherapy on day 22 after the first cycle was initiated. However, up to two weeks of additional recovery time may be provided if medically indicated (delay in neutrophil recovery, infection, or other complication).
h) If a patient develops neutropenia of < 500 cells/µl on 4 or more consecutive biweekly measurements during any chemotherapy cycle administered at dose level 1 or lower, the patient will receive no further induction chemotherapy. At that point, the patient will receive the transplant preparative regimen regardless of the specific CD4 count obtained.

3.2.5.1.1 EPOCH-F/R Dose Adjustments (for Cycles 2 and 3)

a) Rules for dose adjustment are summarized in the following table:
b) Dose adjustments above starting dose level (level 1) consist of a 20% escalation in the daily doses of etoposide, doxorubicin, and cyclophosphamide. Dose adjustments below starting dose level (level 1) consist of a 20% reduction in the cyclophosphamide dose only.
c) Drug doses are based on the duration of ANC nadir during the previous cycles (as determined by consecutive biweekly CBC/differential tests).

If ANC < 500/µl on 1 or fewer measurements	→	Increase 1 dose level above previous cycle.
If ANC < 500/µl on 2 measurements	→	Same dose level as previous cycle.
If ANC < 500/µl on 3 measurements (4 or more measurements if above dose level 1)	→	Decrease 1 dose level below previous cycle.
If ANC < 500/µl on 4 or more measurements, at dose level 1 or lower	→	Discontinue EPOCH-F/R; proceed to transplant conditioning regimen.

3.2.5.1.2 Fludarabine Dose Adjustment

a) If GFR is less than 70ml/min or creatinine ≥ 1.5 mg/dl, Fludarabine dose will be reduced by 20%.

3.2.5.1.3 Doxorubicin Dose Adjustment

a) The doxorubicin component of EPOCH will be omitted in the event that the LVEF is < 45%, as determined either at study entry or during protocol implementation. In addition, doxorubicin may be omitted for additional patients (those with LVEF ≥ 45%) in the event that the ECHO shows evidence of cardiac dysfunction (such as regional hypokinesis).

3.2.5.1.4 Vincristine Dose Adjustment (for EPOCH-F/R cycles 1 to 3):

a) For grade 2 sensory or motor neuropathy reduce vincristine to 50%
b) For grade 3 sensory or motor neuropathy omit vincristine

3.3 TRANSPLANT CHEMOTHERAPY PREPARATIVE REGIMEN (ARM IVD, COHORTS 1 AND 2 ONLY; NOT APPLICABLE TO ARM IVD, COHORT 3)

a) Patients must be at least 22 days from the start of their last cycle of induction chemotherapy. However, in cases where additional recovery time is required (for example, due to prolonged neutropenia, documented infection, or other medical complication), up to two weeks of additional recovery time may be allowed.
b) This low-intensity Flu/Cy preparative regimen will only be given to lymphoma patients on arm IVD who are randomized to cohorts 1 and 2. Patients to be transplanted on arm IVD

cohort 3 (non-lymphoma diagnoses, lymphoma patients under the age of 18 and lymphoma patients with highly progressive disease in combination with severe T cell depletion [CD4 < 100 or ALC < 300]) will not receive the Flu/Cy preparative regimen. Such cohort 3 patients will only receive the high-dose sirolimus prior to transplantation and should be at least 10 days remote from prior chemotherapy. To help ensure engraftment in cohort 3, pre-transplant chemotherapy will be highly immune depleting (resulting in CD4<100 or ALC<300 at the time of pre-transplant sirolimus administration).

c) To proceed with transplantation, the following study inclusion criteria values must be repeated, at the discretion of the PI or LAI, and documented to be within the entry guidelines (Cr < 1.5 mg/dl; T. bili < 2.5 mg/dl; ALT and AST ≤ 2.5 x upper limit of normal). However, if an abnormal value is attributable to malignant disease or cGVHD, such patients will remain eligible for transplantation.
d) Specifics of the transplant chemotherapy preparative regimen are as follows:

3.3.1 Transplant Conditioning Regimen

Drug	Dose	Days
Fludarabine	30 mg/m^2/day IV infusion over 30 minutes	Transplant Days –6, -5, -4, -3
Cyclophosphamide	300 mg/m^2/day IV infusion over 2 hours	Transplant Days –6, -5, -4, -3

3.3.2 Arm IV Transplant Preparative Regimen: Required Hydration

a) For subjects on arm IV that are not capable of following an aggressive hydration regimen of at least four liters per day of oral fluid intake, the following hydration regimen will be initiated just prior to cyclophosphamide infusion (on day –6 of the transplant), and will consist of 0.9% NaCl supplemented with 10 mEq/liter potassium chloride (KCl) at an initial rate of 100 ml/hour. For patients with particularly poor oral intake, the rate of hydration may be increased as clinically indicated. Hydration will continue until 24 hours after the last cyclophosphamide dose has been completed.
b) During hydration, serum potassium level will be monitored every 12 hours. If serum potassium is > 4.5 mEq/l, KCl will be removed from the 0.9% NaCl infusion. If serum potassium is < 3.0, KCl concentration in the 0.9% NaCl will be increased to 20 mEq/l.
c) During hydration, 20 mg of furosemide will be administered daily by IV route to maintain diuresis, with additional doses of furosemide to be given as needed for weight gain due to fluid retention (allow at least four hours of observation between doses).
d) During hydration, if fluid intake exceeds urine output by greater than 500ml during an 8-hour period, an additional 20 mg of furosemide will be administered.
e) For subjects on arm IV that are motivated and capable of following and aggressive hydration regimen of at least four liters per day of oral fluid intake, the low-intensity fludarabine and cyclophosphamide regimen may be administered as an outpatient. In such cases, hospital admission will occur on day -2 at the time of initiation of sirolimus therapy.
f) In such cases of outpatient fludarabine and cyclophosphamide administration, an additional 500 to 1000 ml of 0.9% NaCl fluid bolus should be administered during the cyclophosphamide infusion. Daily serum potassium levels should be monitored during this outpatient therapy,

and KCl infusions should be utilized as necessary to maintain values between 3.0 and 4.5 mEq/l (as described above in section b).

3.4 SIROLIMUS ADMINISTRATION

3.4.1 General Aspects of Sirolimus Administration

a) In the past protocol versions, the protocol specified the time of day for administration of sirolimus to minimize drug interaction with cyclosporine. However, in the current protocol version, sirolimus and cyclosporine are administered sequentially rather than in combination. As such, the time of day for sirolimus administration can be flexible; typically, it will be administered in the morning.
b) Because voriconazole greatly reduces sirolimus clearance via cytochrome p450 inhibition, recipients of sirolimus should not receive voriconazole for the first two weeks post-transplant.
c) Fluconazole has less of an effect on sirolimus clearance, and therefore will be used for fungal prophylaxis in all patients.
d) Based upon a recent publication using this same dosing of sirolimus (52), it is anticipated that the sirolimus drug half-life will be approximately two to three days. This should be considered when making dose adjustments; specifically, when the sirolimus level is above the target, it may be necessary to hold off on the daily dosing until the drug level returns into the therapeutic range.
e) For sirolimus levels, 2.5 cc of blood will be collected into EDTA tubes and analyzed at the NIH Clinical Center.

3.4.2 Arm IVD (cohorts 1, 2, and 3); higher target dose of sirolimus

a) On each cohort on arm IVD, sirolimus will be delivered for a longer period of time pre-transplant than on previous protocol versions. For most patients, sirolimus will be started at day -4 of the transplant; however, for patients with active malignancy who are in need of disease control, sirolimus may be initiated earlier (as early as day -14 post-transplant).
b) Sirolimus will be loaded at a higher dose, 16 mg of Rapamune tablets (rather than 12 mg loading on arm IVB).
c) It is critical that patients have a sirolimus level drawn either at day -2 or day -1 pre-transplant. If the sirolimus level is subtherapeutic (< 20 ng/ml), consideration should be given to re-bolus sirolimus in order to achieve the desired drug level by the time of infusion of the allogeneic hematopoietic cell product.
d) Sirolimus maintenance dosing will be Rapamune tablets, 4 mg, p.o., each day. Sirolimus dose should be adjusted to maintain therapeutic concentrations in the range of 20 to 30 ng/ml, with sirolimus levels drawn on a Monday, Wednesday, Friday schedule (if inpatient) or a Monday, Thursday schedule (if outpatient).
e) Because the sirolimus steady-state level will be maintained at a higher level (20 to 30 ng/ml), the sirolimus will be discontinued earlier on arm IVD, with discontinuation of sirolimus at day 7 post-transplant (sirolimus was administered until day 14 post-transplant on arm IVB).

3.5 CYCLOSPORINE GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS (ALL PATIENTS ON ARM IVD)

a) For arm IVD, cyclosporine will be initiated on day +7 after the transplant (at the time of discontinuation of sirolimus). This differs from previous arms (such as arm IVC), where cyclosporine was initiated at day -4 of transplant. The rationale for moving the cyclosporine to day 7 post-transplant is as follows: (a) limitation of toxicity, such as renal insufficiency (concomitant sirolimus/cyclosporine vs. sequential sirolimus ➔ cyclosporine); and (b) the low rate of acute GVHD observed on protocol 08-C-0088, which utilized single-agent high-dose sirolimus .
b) cyclosporine will be administered by oral route at an initial dose of 2 mg/kg/dose every 12 hours; however, in unanticipated cases where oral therapy is not possible, IV cyclosporine infusion will be given at 2 mg/kg/dose every 12 hours over a 2-hour period. Subsequently, doses will be adjusted according to trough levels monitored biweekly and/or upon symptoms or alteration in renal function.
c) The target range for serum cyclosporine levels will be 200–250 μg/ml. After day +100, provided acute GVHD is grade 0 or I, cyclosporine will be tapered by approximately 10% from the last dose administered each week to a dose of 25 mg/day. Cyclosporine will then be completely discontinued if there are no signs of GVHD.
d) In the event of malignant disease progression before day 100, early taper or discontinuation may be warranted (decision granted by PI and LAI).

3.6 TRANSPLANT PROCEDURE: ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION OR BONE MARROW TRANSPLANTATION

a) On day 0, the patient will receive the cryopreserved PBSC or marrow cells.
b) The cryopreserved product will be thawed and immediately administered intravenously. The target dose of the PBSC is $\geq 4 \times 10^6$ $CD34^+$ cells per kg. However, if donor apheresis on days 5, 6, and 7 yields a total of $\geq 3 \times 10^6$ $CD34^+$ cells per kg, this level of $CD34^+$ cell dose will also be allowed. The upper range of CD34 dose should be 8×10^6 cells per kg.
c) If cell transfer results in DMSO-related toxicities (chills, muscle aches), diphenhydramine and meperidine may be administered, but steroids are not allowed.

3.7 TRANSPLANT PROCEDURE: DAY 14 PRE-EMPTIVE DLI

a) For patients treated on arm IVD cohorts 1 and 2, cryopreserved donor Th2 cells or unmanipulated donor T cells will be thawed and immediately administered intravenously on day +14 post-transplant. Cohort 3 will not be randomized, and will receive the Th2 cells pre-emptively on day 14 post-transplant.
b) The dose of Th2 cells or unmanipulated donor T cells will attempt to be held constant for each study recipient (target dose 2.5×10^7 Th2/kg; minimum dose will be 1×10^7 Th2/kg).
c) No steroids will be allowed for management of DMSO-related toxicities (chills, muscle aches) that may occur after cellular infusion (diphenhydramine and meperidine are allowed).
d) Determination of whether a Th2 cell infusion is safe will be based on the presence or absence of hyperacute GVHD, grade 4 or 5 toxicity attributable to the Th2 cells, and whether the alloengraftment goal has been achieved.
e) For this study, hyperacute GVHD will be defined as grade III or IV acute GVHD that occurs within the first 14 days after Th2 cell infusion.

f) In the unexpected event that a patient develops a serious post-transplant toxicity in the first 14 days post-transplant (particularly, engraftment syndrome), then the patient may be managed by one of two methods (specific method will be determined by the PI and Lead AI). In some cases (method 1), the patient will be deemed not eligible to receive the DLI (for example, a patient with engraftment syndrome requiring ongoing steroid therapy in the setting of predominant donor chimerism). In such a case, the patient will be removed from the randomization or from the cohort 3 therapy and a "replacement patient" will be accrued. For the patient with the unexpected event, the toxicity will be managed and further post-transplant care will be provided on protocol according to standard practices. Alternatively (method 2), the patient will be allowed to recover from the early post-transplant toxicity and the pre-emptive DLI may be administered in a delayed manner (up to day 35 post-transplant instead of the planned day 14 post-transplant). Method 2 would be chosen in cases where the toxicity is taking some time to resolve and in the setting of mixed chimerism or persistent tumor (cases where additional DLI are clinically indicated).
g) In the unexpected event that the experimental T cell product is not available for administration on day 14 post-transplant, then the product may be administered in a delayed manner when the product is available. Specific reasons that may account for a delay in T cell availability include T cell manufacturing insufficiencies.

3.8 TRANSPLANT PROCEDURE: GROWTH FACTOR ADMINISTRATION POST-TRANSPLANT (FOR ALL PATIENTS ON ARM IVD, COHORTS 1, 2, AND 3)

a) Because the low-intensity chemotherapy is associated with either minimal or no neutropenia, recombinant human filgrastim will not be routinely administered.
b) In the event that significant neutropenia does develop post-conditioning (< 200 ANC for three days), then Filgrastim will be administered at a dose of 5 ug/kg/day s.c., typically until the ANC is greater than 5000 cells per ul (discontinuation of filgrastim may occur earlier than this value if approved by the PI or Lead AI).

3.9 TREATMENT OF PERSISTENT DISEASE POST-TRANSPLANT: DLI AND OTHER THERAPY

a) Patients with persistent or progressive malignant disease post-SCT will be eligible to receive donor lymphocytes ("delayed lymphocyte infusion" or DLI) as a component of this study. DLI may be administered alone or after chemotherapy administration. Additional DLI may consist of donor Th2 cells, if available (dose range, 1 to 2.5 x 10^7 Th2 cells/kg); alternatively, DLI may consist of donor cell products collected either in the steady-state or after G-CSF mobilization.
b) Donor lymphocytes will be collected by apheresis, either in steady state (no donor therapy) or after G-CSF mobilization. The donor product may be enriched for lymphocytes by Ficoll-Hypaque procedure as per NIH DTM protocol or by established methods at Hackensack. Alternatively, in cases where additional donor stem cells are desired, the donor product may be administered without lymphocyte purification. DLI may be sequentially administered, with initial dosing typically at 5 x 10^6 or 2 x 10^7 $CD3^+$ T cells per kg, with subsequent dose increases to 1 x 10^8 T cells per kg. In the event that there are excess donor lymphocytes after aliquoting cells into clinical dosing units for patient use, i.e., 5-10x10^6 CD3+ cells/kg, the extra cells, up to 10 percent of the collection, may be used for research.
c) Alternatively, persistent or progressive disease may be treated with any approved therapy thought to be in the best standard care of the patient, such as chemotherapy, cytokine therapy,

radiation therapy, or monoclonal antibody therapy. Alternatively, patients with relapse may receive therapy on other NCI protocols. As described below in the data management section, data collection after standard care therapy will not be protocol-driven but rather will be on a case-by-case basis as determined by the PI/LAI, to be discussed at weekly protocol meetings.

3.10 INTERIM EVALUATION DURING INDUCTION CHEMOTHERAPY

The following studies will be obtained after each cycle of EPOCH-F/R:
1) Routine chemistry and hematology panels.
2) Lymphoma patients: CT scans (chest, abdomen, pelvis, and neck, if indicated).
3) Myeloma patients: serum protein electrophoresis, serum Ig level, 24h collection of urine for urine protein electrophoresis, B_2 microglobulin, immunofixation if M protein is undetectable, bone marrow biopsy.

3.11 PRE-TRANSPLANT EVALUATION

The following studies will be obtained within 28 days of transplantation:
1) Routine chemistry and hematology panels;
2) Bone marrow aspiration and biopsy (as clinically indicated, at the discretion of the PI/LAI)
3) For patients with lymphoma or CLL: CT scans of chest/abdomen/pelvis (and neck, if indicated); FDG-PET scan (if indicated).
4) For patients with multiple myeloma, consider the utility of the following tests: serum protein electrophoresis, serum Ig level, 24h collection of urine for urine protein electrophoresis, B_2 microglobulin; immunofixation (if M protein undetectable); bone marrow biopsy; skeletal survey. The selection of the specific tests will be determined by the PI or LAI, as clinically indicated for any individual patient.
5) Urine β-HCG for women of childbearing potential.

3.12 DETERMINATION OF DONOR/HOST CHIMERISM POST-TRANSPLANT

a) Determination of donor vs. host chimerism will be performed by VNTR-PCR method by a CLIA-certified lab (can be performed either at NIH or at HUMC).
b) Initial baseline determination on donor and recipient will be at time of study entry.
c) Post-transplant chimerism will be determined at the following time points (+/- 7 days):
    1) day 14 post-transplant will evaluate T lymphoid, myeloid, and total blood chimerism.
    2) Blood chimerism will also be evaluated at day 28, day 60, day 100, and 12 months post-transplant. Marrow chimerism may be tested at day 28 post-BMT, and at other time points, but these tests will be performed only as clinically indicated, as determined by the PI/LAI (i.e., mixed chimerism or suspicion of relapse).
    3) If the day 14 chimerism shows > 95% donor elements, these later time points will only evaluate total chimerism.
    4) Chimerism may be measured at other time points, if indicated (e.g., to determine effect of immune modulation in attempt to increase donor chimerism).
    5) In the event that a patient must receive standard of care therapy to treat a transplant complication such as progressive malignancy or graft rejection, then it is likely that this time course for chimerism will not be optimal. As such, in these cases, chimerism tests will be performed as clinically indicated, as determined by the PI/LAI at weekly protocol meetings.

3.13 CYCLOSPORINE MONITORING

a) Blood samples will be sent for measurement of CSA level. This test will be performed once per week through day 100 post-BMT. Steady state trough level for CSA should be maintained in the range of 200 to 250 ng/ml.
b) Additional blood CSA levels will be sent as clinically indicated (i.e., occurrence of nausea, vomiting, headaches, hypertension, or increase in creatinine).

3.14 POST-HSCT EVALUATION

a) After completion of therapy the patient will be followed for potential complications related to allogeneic SCT. The patient will be followed at least once per week for the first 28 days post-transplant; after day 28 and through day 100, the patient may be seen either at the NIH Clinical Center or at their referring center.
b) The patient will be seen in follow-up to evaluate disease status and late problems related to allogeneic HSCT, at days +28(+/- one week) and +100(+/- 2 weeks); and at 6, 9, 12, 18, and 24 months post-transplant (+/- one month). At these times patients will have the following tests performed to determine clinical response:
    1) Routine chemistry and hematologic panels;
    2) If clinically indicated to monitor disease, bone marrow aspiration and biopsy (marrow study may include flow cytometry, cytogenetics, or molecular studies; in general, each patient should have bone marrow examination at day 100 post-transplant (+/- 7 days) (but evaluation of marrow at day 28 post-transplant will not be required).
    3) For patients with lymphoma: CT scans of chest/abdomen/pelvis (and neck, if clinically indicated). FDG-PET scan will be obtained 28 and 100 days post-transplant (+/- 7 days) in all patients with lymphoma (if clinically indicated). For patients who enter into CR or CRu with negative PET, follow-up evaluation will include PET at 6 and 12 months post-transplant. Other time points may be included if clinically indicated.
    4) For patients with multiple myeloma: Consider serum protein electrophoresis with M protein; serum Ig level; 24h collection of urine for urinary protein excretion, protein electrophoresis and M protein; B_2 microglobulin; immunofixation if M protein is undetectable; all tasks will be performed as clinically indicated.

3.15 CONCURRENT THERAPIES

a) Infectious disease prophylaxis as defined below, in Section **4**.

3.16 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.16.1 Criteria for removal from protocol therapy

1) Completion of protocol therapy
2) Patient requests to be withdrawn from protocol therapy
3) Initiation of additional or alternative standard of care treatments
4) Investigator discretion

5) Positive pregnancy test

3.16.2 Off Study Criteria

The donor or recipient will be removed from protocol for any of the following reasons:
1) Unacceptable toxicity (> grade 3) for the donor.
2) Irreversible non-hematologic toxicity (> grade 3 of 14 day duration) for the recipient while receiving induction chemotherapy (EPOCH-F/R).
3) The donor or recipient refuses to continue therapy.
4) In addition, the patient may at any time be removed from protocol at the principal investigator's discretion, if the PI deems the patient to be at unacceptable risk to remain on study. Reasons for this action may include (but are not limited to) disease progression with declining organ function/performance status before transplantation; inadequate family/caregiver support; noncompliance.
5) PI decision to end this study
6) Donor subjects who become decisionally impaired
7) Death

3.17 OFF STUDY PROCEDURE

3.17.1 NCI

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

3.17.2 Participating Sites

The Participant Status Updates Form will be supplied by the CCR study coordinator. Send the completed form to the CCR study coordinator.

4 BIOSPECIMEN COLLECTION

4.1 IMMUNE LAB STUDIES: PRE-TRANSPLANT INDUCTION CHEMOTHERAPY CYCLES

a) Blood samples will be drawn to evaluate the effects of induction chemotherapy on host immune depletion (3 cc lavender tube to NIH Clinical Pathology for Lymphocyte Phenotyping - TBNK; 20 cc in heparinized green top tube to Dr. Fran Hakim, Building 10, 12th Floor Pre-Clinical Core).

b) This sample should be drawn just prior to each cycle of induction chemotherapy (within six days of the next cycle).

4.2 IMMUNE LAB STUDIES: EVALUATION OF TRANSPLANT CHEMOTHERAPY PREPARATIVE REGIMEN

a) Blood samples will be drawn to evaluate the effects of the fludarabine and cyclophosphamide regimen on host immune depletion. Send 3 cc lavender tube to NIH Clinical Pathology for

Lymphocyte Phenotyping - TBNK; send 20 cc in heparinized green top tube to Dr. Fran Hakim, Building 10, 12th Floor Pre-Clinical Core.

b) Timepoints for this aspect of the study will be: 1) immediately prior to preparative regimen chemotherapy (day –6); and 2) just prior to the PBSCT (day 0).
c) Samples should be delivered to Dr. Hakim (Bldg 10, 12th Floor Pre-Clinical Core). Experiments will consist of flow cytometry to detect host lymphoid depletion.

4.3 IMMUNE LAB STUDIES: EVALUATION OF TYPE I VERSUS TYPE II CYTOKINE EFFECTS POST-TRANSPLANT

a) Blood will be drawn for cytokine evaluation. (This will be collected at NIH as two 8ml CPT tubes and one 8 ml serum separator tube, and at HUMC as four 10ml green top heparinized tubes and one 8 ml serum separator tube due to supply availability.) Samples will be delivered to Dr. Hakim (Bldg 10, 12th floor pre-clinical core). Samples collected at Hackensack will either be shipped to NIH fresh or after cryopreservation.
b) Cytokine evaluation at the NIH and Hackensack will occur on days 7, 14, and 28 post-transplant (+/- 3 days) and at 2 and 3 months post-transplant. Samples collected at Hackensack will be drawn and then shipped the same day to Dr. Hakim.

4.4 IMMUNE LAB STUDIES: EVALUATION OF IMMUNE RECONSTITUTION POST-TRANSPLANT

a) For subjects treated at NIH and Hackensack, blood will be sent to Fran Hakim (Bldg 10, 12th Floor Pre-Clinical Core) for evaluation of immune reconstitution post-transplant. (This will be collected at NIH as five 8ml CPT tubes, and at HUMC as six 10ml green top heparinized tubes due to supply availability.) These samples will be sent on day 7, 14, and 28 post-transplant (+/- 3 days), at 2 and 3 months post-transplant, and then every 3 months for the first year, every 6 months for the second year, and then yearly until 5 years post-transplant. For patients that receive the investigational Th2 cells as a treatment of persistent disease post-transplant, these immune reconstitution labs may be increased to a frequency not to exceed once per month to allow an investigation of the effect of delayed Th2 cell infusion on immune reconstitution.
b) Immune studies will also be performed on bone marrow aspirate samples; as described previously, bone marrow aspirate procedures will be performed only as clinically indicated. One aspirate will be sent for clinical tests; one additional aspirate will be sent to Dr. Hakim's lab for research analysis of marrow infiltrating lymphocyte populations.
c) In some cases, it may be necessary to biopsy tissue post-transplant to confirm a diagnosis of malignant disease relapse. In such cases, a portion of the biopsy sample will be sent to Dr. Hakim's lab for research analysis of tumor infiltrating lymphocyte cells.

4.5 IMMUNE LAB STUDIES: EVALUATION OF IMMUNE CELL POPULATIONS AND MOLECULAR EVENTS IN THE TARGET TISSUES OF CHRONIC GVHD

a) Buccal mucosal, minor salivary gland (lower lip), and skin biopsy samples collected on subjects undergoing clinically indicated biopsies to diagnose GVHD will be used to evaluate immune cell populations and cytokine profiles associated with the development of chronic GVHD (a portion of each biopsy will be sent to Gress Lab 12C121).
b) The pathogenesis of chronic GVHD is poorly understood and no studies rigorously evaluated cellular and molecular changes that occur in the target tissues of chronic GVHD. Heterogeneity in patient and treatment related factors complicate the group comparisons.

Prospectively designed study with planned sequential sample collection is the ideal format that would give the best chance of success in study of this complex problem.

c) We will focus on the skin and oral cavity as the two most common target organs of chronic GVHD. An optional biopsy consisting of a standard 6 mm punch biopsies will be performed in the skin and buccal mucosa in patients at the time of initiation of immunosuppressive taper prior to onset of clinical chronic GVHD at day 63+/-3. Another optional biopsy will be performed at the onset of clinical chronic GVHD and at 6 months post transplant in patients who do not develop chronic GVHD by this time point. Whole saliva will be collected at the same time as the buccal mucosal biopsies are performed to evaluate the changes in the salivary proteome at the onset of chronic GVHD. The samples will be analyzed using a variety of methods including immunofluorescence and confocal microscopy, gene expression profiles, and protein based assays in order to better understand the reconstitution of resident immune cell populations following HSCT and how they change with the onset of chronic GVHD (samples to Gress Lab 12C121).

4.6 COLLABORATIVE RESEARCH STUDIES:

4.6.1 Isolating novel human antibodies (completed as of 01/01/2016)

Under Amendment EE, coded serum samples and Human Peripheral blood Mononuclear Cells will be sent to the Scripps Research Institute to isolate novel human anti-B-cell maturation antigen (BCMA) antibodies that could be used as monoclonal antibody or chimeric antigen receptor (CAR) therapies for human **cancer.**

Rationale: Dr. Rader is a recognized expert in isolating novel human antibodies by using human peripheral blood mononuclear cells (PBMC) and human serum from post-allogeneic-transplant patients.

Specific Aims: To isolate novel human anti-B-cell maturation antigen (BCMA) antibodies that could be used as monoclonal antibody or chimeric antigen receptor (CAR) therapies for human cancer.

Design/Procedures:

The samples will be sent to laboratories at the Scripps research institute to isolate novel human anti-B-cell maturation antigen (BCMA) antibodies after an MTA is executed between the NCI and The Scripps Research Institute. All excess samples will be destroyed at the conclusion of the planned studies. The serum and human peripheral blood mononuclear cells (PBMC) samples will be sent to the following address:

Christoph Rader PhD

The Scripps Research Institute

130 Scripps Way #2C1

Jupiter, FL 33458

Coded serum samples will be sent to Dr. Rader from patients participating in the 04-C-0055 clinical protocol at NCI. Cryopreserved heparinized serum from up to 10 patients will be transferred through this collaboration.

Laboratory results will be sent back to Dr. Fowler for data compilation and analysis. Dr. Rader will not have access to patient identifiers or patient information.

Only the subject's unique identifier will be used and information about the enrolling site will be removed. Authorized investigators at NCI and Prof. Dr. Rader will maintain codes linking the laboratory specimens received to clinical information; however, Dr. Rader will not have access to any other patient identifiers or patient information. Sample identifiers are not derived from actual identifiers and Dr. Rader research team will not have access to the re-identification key.

5 SUPPORTIVE CARE

5.1 INFECTION PROPHYLAXIS (ALSO, SEE APPENDIX A: PROPHYLAXIS AND TREATMENT OF INFECTIOUS COMPLICATIONS)

a) All patients will receive prophylaxis against *Pneumocystis carinii* pneumonia, beginning with the first cycle of EPOCH-F/R induction therapy, continuing until transplantation, and resuming at the time of platelet recovery. PCP prophylaxis will continue until day +100 or until immunosuppression is discontinued. Trimethoprim/sulfamethoxazole is the preferred regimen.
b) All patients will receive fluconazole for prophylaxis against yeast infections. Because of its interaction with vincristine, fluconazole should not be taken concurrently with EPOCH-F/R chemotherapy. Therefore, fluconazole will start on day 6 of the first cycle of pre-transplant induction chemotherapy and will be held on days 1 through 5 of each subsequent cycle. Fluconazole will continue post-transplant until immunosuppression is discontinued.
c) Antibiotic therapy for neutropenic patients will be managed in accordance with CDC guidelines. In cases where neutropenia and fever persist for longer than 5 days despite broad-spectrum antibiotics, empiric antifungal therapy with liposomal amphotericin B or with voriconazole should be considered.
d) All patients will receive valacyclovir for prophylaxis against herpes simplex and varicella zoster virus infection/reactivation. This therapy will start on day 1 of pre-transplant induction therapy, continuing through transplantation until day +100 or until immunosuppression is discontinued.
e) Patients with positive pre-transplant serology for Cytomegalovirus (CMV) and/or CMV-seropositive donors will be monitored for CMV reactivation by weekly testing for CMV PCR and clinical evaluation. Positive PCR will be treated according to the schema in Appendix A. Weekly monitoring will continue through day +100, or longer for patients at risk.
f) Patients receiving immunosuppression for chronic GVHD will receive penicillin V for prophylaxis against bacterial infections. All patients will undergo vaccination against *S. pneumoniae* and *H. influenzae*, beginning 12 months after transplantation.

5.2 MANAGEMENT OF ENGRAFTMENT SYNDROME

Engraftment syndrome may occur at the time of neutrophil recovery. Its clinical manifestations include noninfectious fever, rash, and vascular leak causing noncardiogenic pulmonary edema, weight gain, and renal insufficiency. See Appendix B for engraftment syndrome diagnostic criteria and treatment schema.

5.3 TREATMENT OF GRAFT-VERSUS-HOST DISEASE

a) In patients in whom GVHD is suspected, standard clinical criteria and biopsy findings (when clinically indicated) will be used to establish the diagnosis. Acute GVHD will be graded by the Glucksberg criteria (Appendix C) and according to the 2005 NIH consensus guidelines (see Appendix E).
b) Patients with clinical Stage 1 or 2 (Grade I) GVHD of the skin without any other organ involvement will be treated with a topical corticosteroid cream.
c) In general, patients with ≥ Grade II acute GVHD will be treated with high-dose, systemic corticosteroids.
d) Patients who fail to respond to corticosteroids will be considered for second-line immunosuppressive therapy, e.g., tacrolimus, mycophenolic acid, monoclonal antibodies, or other experimental acute GVHD protocols, if they are available.

5.4 MENSES SUPPRESSION AND CONTRACEPTION

a) At the time of study entry, pre-menopausal women who have not undergone hysterectomy should be given the option to be placed on medroxyprogesterone acetate 10 mg/day orally to suppress menses. This therapy will be continued until platelet recovery after transplantation (> 50,000/mm^3 without transfusion). However, because most patients on low-intensity transplantation do not have this level of thrombocytopenia, an option to avoid use of this drug can be provided to the patient.
b) Female transplant recipients will be advised to use contraception for at least 1 year after transplantation and to have their male partners use condoms.
c) Male transplant recipients will be advised to use contraception, preferably condoms, for 1 year after transplantation.

5.5 BLOOD PRODUCT SUPPORT

a) Patients will receive packed red blood cells and platelets as needed to maintain Hb > 8.0 gm/dl, and plts > 10,000/mm^3 (or higher, if clinically indicated).
b) All blood products, with the exception of the stem cell product and the Th2 cell product, will be irradiated.
c) Leucocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused leukocytes and decrease the risk of CMV infection.

5.6 NUTRITIONAL SUPPORT

a) If mucositis or GVHD prevents adequate PO intake, parenteral hyperalimentation will be instituted and discontinued under the direction of the dietary service.
b) Oral intake will resume when clinically appropriate under the supervision of the dietary service.

5.7 ANTI-EMETICS

Anti-emetic usage will follow Clinical Center Guidelines or Hackensack University guidelines as well as recommendations from the Pharmacy services.

5.8 INTRAVENOUS IMMUNE GLOBULIN (IVIG)

a) Will follow Guidelines for Infection Management in Allogeneic HSCT Recipients by the NIH Blood and Marrow Consortium or Hackensack standard practices.
b) IgG level will be measured only in patients who have had ≥ 2 respiratory infections that required antibiotic therapy over the past six months.
c) For IgG values less than 400mg/dl, 500mg/kg IVIG will be administered intravenously.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

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 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.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

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.

a) Data will be prospectively collected and entered into the Cancer Central Clinical Data System database (C_3D; information at http://ccrtrials.nci.nih.gov).
b) For more information about data collection please see Appendix D.
c) For this study, the development of hyperacute GVHD will be considered a toxicity likely attributable to Th2 cell administration. Hyperacute GVHD will be defined as severe GVHD (grade III or IV) that occurs in the first 14 days after Th2 cell infusion.
d) Data collection, including adverse events, will not be required for the standard care therapy administered prior to the preparative chemotherapy (conditioning) and transplant or after standard care therapy to treat progressive disease.

6.2 RESPONSE CRITERIA

6.2.1 Complete Response (CR)

6.2.1.1 For non-Hodgkin's lymphoma or Hodgkin's lymphoma:

a) Complete disappearance of all detectable signs and symptoms of lymphoma for a period of at least one month.
b) All lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in greatest transverse diameter for nodes > 1.5 cm before therapy).

c) Previously involved nodes that were 1.1 to 1.5 cm in greatest transverse diameter before treatment must have decreased to ≤ 1 cm in their greatest transverse diameter after treatment or by more than 75% in the sum of the products of the greatest diameters (SPD).
d) In the event that the spleen or other organ is enlarged due to lymphoma involvement prior to therapy, organ must regress in size by CT scan and must not be palpable on physical examination. Any macroscopic nodules in any organs detectable on imaging techniques should no longer be present.
e) If bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of the same site.

6.2.1.2 For chronic lymphocytic leukemia (CLL):

Complete resolution of detectable signs and symptoms for at least 2 months, with peripheral blood lymphocytes ≤ 4K/µl, neutrophils ≥ 1.5K/µl, platelets ≥ 100K/µl, hemoglobin > 11g/dl (untransfused), bone marrow lymphocytes < 30% without lymphoid nodules.

6.2.1.3 For chronic myelogenous leukemia (CML):

a) Hematologic CR: a) normalization of peripheral blood counts (WBC < 10K/µl, platelets < 450K/µl); b) no immature cells on peripheral smear (blasts, promyelocytes, metamyelocytes).
b) Cytogenetic CR: a) must meet aspects of hematologic CR, and; b) cytogenetic studies negative for Philadelphia chromosome (Ph).
c) Molecular CR: a) must meet aspects of hematologic and cytogenetic CR, and; b) PCR studies negative for *bcr/abl*.
d) For each of above determinations, remission status must be maintained for at least one month.

6.2.1.4 Other leukemias, myeloproliferative disorders, or myelodysplastic syndrome:

a) Normalization of peripheral blood counts and bone marrow morphology, with fewer than 5% blasts in bone marrow.
b) Absence of specific molecular or cytogenetic markers of disease.
c) In the case of myeloproliferative disorders, there must be absence of bone marrow fibrosis with normal hematologic parameters.
d) Remission status must be maintained for at least one month.

6.2.1.5 Multiple Myeloma:

a) Absence of urine and serum M-components by immunofixation and electropheresis (normalization of immunoglobulins is not required).
b) Clonal plasma cells in bone marrow <1% by PCR or flow cytometry.
c) Normal serum calcium.
d) No new bone lesions; no enlargement of existing lesions.
e) Vertebral collapse or other pathological fracture due to osteoporosis or existing lesion does not prevent categorization as CR.
f) Remission status must be maintained for at least one month.

6.2.2 Complete Response/unconfirmed (CRu)

a) In cases of non-Hodgkin's lymphoma or Hodgkin's lymphoma, a residual lymph node mass > 1.5 cm in greatest transverse diameter will be considered CRu if it has regressed by more

than 75% in the SPD, does not change over at least one month, is negative by PET or gallium, and is negative on any biopsies obtained (biopsy not required).
b) Individual nodes that were previously confluent must have regressed by more than 75% in their SPD compared with the size of the original mass and be stable for at least one month.
c) Any residual lesions in involved organs must have decreased by more than 75% in the SPD or be < 1 cm, be clinically consistent with residual scarring, and be stable for at least one month.
d) Indeterminate bone marrow, if previously involved with lymphoma, will also be considered CRu.

6.2.3 Partial Response (PR)

a) For non-Hodgkin's lymphoma or Hodgkin's lymphoma: a) a 50% or greater decrease in SPD of all measured lesions lasting for a period of at least one month, and; b) no individual lesion may increase in size, and no new lesions may appear.
b) For chronic lymphocytic leukemia: a) a 50% or greater decrease in SPD of measured lymph nodes, hepatomegaly, or splenomegaly lasting at least 2 months, and; b) one or more of the following: neutrophils $\geq$ 1.5K/µl, platelets > 100K/µl, or hemoglobin > 11g/dl (or 50% improvement).
c) For chronic myelogenous leukemia: a) to qualify as "hematologic PR", must have same features as hematologic CR, except for (1) persistence of immature cells, or (2) platelets < 50% pretreatment level but > 450K/µl, or (3) persistent splenomegaly but < 50% of pretreatment size; b) to qualify as "cytogenetic PR", must have same features as hematologic CR, with only 1-34% Ph-positive cells (major response); c) to qualify as "cytogenetic minor response", must have hematologic CR, with 35-90% Ph-positive cells.
d) For multiple myeloma: a) reduction by $\geq$ 75% in serum myeloma protein production, with decrease in Bence-Jones proteinuria by $\geq$ 90%; b) clonal marrow plasmacytosis $\leq$ 5%; c) no new lytic bone lesions.

6.2.4 Stable Disease (SD)

a) For non-Hodgkin's lymphoma or Hodgkin's lymphoma: tumor measurements not meeting the criteria for CR, CRu, PR, or PD.
b) For CLL: response parameters not meeting criteria for CR, PR, or PD.

6.2.5 Relapsed or Progressive Disease (PD)

a) For NHL or Hodgkin's lymphoma: $\geq$ 25% increase in SPD of all measured lesions compared to smallest prior values, or appearance of any new lesion(s).
b) CLL: $\geq$ 50% increase in SPD of all measured lesions compared to smallest prior values; new lesion(s) or $\geq$ 50% increase in blood lymphocytes; Richter's syndrome.
c) CML: Increase in the number of metaphases demonstrating Ph by cytogenetics or t(9:22) by FISH; return to PCR^+ for *bcr/abl* after previously becoming negative.
d) Other leukemias, myeloproliferative disorders, or myelodysplastic syndrome: marrow and blood morphological features consistent with relapse or progression, rising blast count, or re-emergence of specific molecular or cytogenetic markers.
e) Multiple Myeloma (requires 2 of the following): increase in serum M-protein to $\geq$ 50% above lowest level or rise $\geq$ 2 g/dl; increase in urine light chain excretion to 50% above the

lowest value (at least 250 mg/24 hours) or an increase ≥ 2 g/24 hours of light chain excretion; increase in soft tissue plasmacytomas by 50% or new or increasing lytic bone lesions; the above protein criteria for relapse, plus hypercalcemia > 12 mg/dl, anemia with hemoglobin decrease > 2 g/dl, increased bone marrow plasma cells by 50%, or generalized bone pain.

6.2.6 Refractory Disease

Disease progression at any time during treatment with EPOCH-F/R therapy, or failure to achieve CR or PR after EPOCH-F/R and/or allogeneic HSCT.

6.3 TOXICITY CRITERIA

The NCI Common Terminology Criteria for Adverse Events version 3.0 will be used (CTCAEv3). This document can be found at: http://ctep.info.nih.gov/CTC3/default.html

7 STATISTICAL CONSIDERATIONS

a) Protocol version amendment E introduced a new statistical section. This amendment allowed further accrual to the only actively accruing cohort (arm IV) and opened a new arm (arm V) designed to evaluate whether alloengraftment can be consistently achieved without administration of the protocol preparative regimen. Arm IV involves allogeneic HSCT using a significantly reduced preparative regimen (reduction in total cyclophosphamide dose from 4800 mg/m^2 to 1200 mg/m^2) and day 14 post-HSCT infusion of donor Th2 cells. As such, feasibility of transplantation without a preparative regimen will be an underlying goal of arm V.
b) Arms I, II, and III of the prior protocol version, which used the high-dose preparative regimen (cyclophosphamide dose, 4800 mg/m^2) will continue to be closed to accrual.
c) The decision to treat an enrolled subject on arm IV (transplantation with a preparative regimen) or on arm V (transplantation without a preparative regimen) will initially be based upon the subject's CD4$^+$ T cell count at the time of study entry and after completion of cycle 1 and 2 of protocol induction chemotherapy with EPOCH-FR.
d) In the current protocol implementation (version J), newly enrolled subjects with an entry CD4 count < 200 cells per microliter or protocol subjects with a CD4 count of < 200 cells per microliter after cycle 1 or cycle 2 of EPOCH-F chemotherapy are assigned to transplantation on arm V, and therefore receive one additional cycle of EPOCH-F therapy and HSCT with Th2 cell therapy on day 0 of the transplant. At this stage of protocol implementation (12/17/2007), 20 out of a maximum of 25 patients have been transplanted on arm V.
e) In the current protocol implementation (version J), all subjects who do not achieve the CD4 target of < 200 cells/µl after 2 cycles of EPOCH-F will be assigned to transplantation on arm IV, and therefore will receive a third cycle of EPOCH-F, the transplant preparative regimen (fludarabine combined with cyclophosphamide [total dose, 1200 mg/m^2]), and HSCT with delayed infusion of Th2 cells (day 14 post-HSCT). At this stage of protocol implementation (12/17/2007), only 8 out of a maximum of 25 patients have been transplanted on arm IV.
f) In amendment version K, to correct for the imbalance between arm V vs. arm IV patient allocation (n=20 vs. n=8, respectively), patient allocation will be changed once the goal of n=25 subjects on arm V has been attained. At such a point: (1) no further patients will be transplanted on arm V; (2) all patients will be transplanted on arm IV after the low-intensity Flu/Cy preparative regimen, with Th2 cells administered on day 14 post-transplant; (3) the timing of arm IV transplantation will be after one or two cycles of EPOCH-F chemotherapy

(provided that the CD4 count is < 200) or after a maximum of three cycles of EPOCH-F therapy (as is currently being performed). Any analyses regarding engraftment performed using data from arm IV will be done after comparing results from patients enrolled in arm IV prior to the modification outlined in amendment K versus those enrolled in arm IV after amendment K. This will be done to ensure that the patients in arm IV have sufficiently homogenous outcomes with respect to engraftment to permit them to be considered one overall group for analysis.

g) For arm V, the goal will be to determine if >= 23 of 25 total patients enrolled on have satisfactory allo-engraftment. Satisfactory alloengraftment will be defined as the attainment of > 50% donor T cell chimerism by day 100 post-transplant. If the true underlying probability of an individual patient having such success were 0.85, then the probability of have 23-25 is 0.098. On the other hand, if the true underlying probability of an individual patient having such success were 0.98, then the probability of having 23-25 is 0.783. Thus, obtaining 23+/25 with a satisfactory outcome using this cascading enrollment strategy provides moderately strong evidence that the true probability of success is nearly 100% and unlikely to be as low as 85%.

h) At any point during protocol implementation, if failure to meet the engraftment endpoint is observed in two arm V subjects (out of a maximum of 25 subjects), then accrual to arm V will be stopped. Furthermore, if the PI and research team has other concern, either anticipated or not anticipated, relating to the quality of engraftment observed, then continuation of further accrual at a lower arm V level will be warranted and permitted.

i) With amendment E, a new study objective will be to compare the allo-engraftment results from arm IV and arm V subjects. Because of this change in study objective from acute GVHD to allo-engraftment, accrual to arms IV and V will proceed in a continuous manner, and not according to the two-stage optimal design utilized in the prior protocol version. Both arm IV and arm V are anticipated to have very high rates of engraftment, likely in excess of 90%. Thus, the objective will be to compare the rates of engraftment, keeping in mind that a statistically significant difference would not be anticipated, that eligibility for enrolling in arm IV vs. V shifts as patients are enrolled, and that the study will have too few patients to permit a reasonable non-inferiority comparison. The final analysis will be descriptive and comment on the similarities and differences between the arms, including taking into consideration the CD4 counts of patients enrolled at each cohort and overall.

j) Under amendment E, the accrual ceiling to arm IV increased slightly to 25, to equal that of arm V, with a new goal of establishing the engraftment rate on arm IV, for comparison to arm V. With 25 patients enrolled on arm IV, if the true probability of engraftment were 0.85, then the probability of seeing 23-25 of 25 engrafting is 0.25 (and of seeing 24-25 of 25 engrafting is 0.093), while if the true probability of engraftment were 0.98, then the probability of seeing 23-25 engrafting is 0.987 (and the probability of seeing 24-25 of 25 engrafting is 0.91). Thus, the probability of having very high rates of engraftment on arm IV is high if the true individual probability is nearly 100% and very low if the true individual rate is only 0.85.

k) Under amendment E, for safety reasons, an unacceptably high rate of grade II to IV acute GVHD (defined by more than a 50% incidence) will be utilized as a stopping rule for arm IV and arm V. The rate of acute GVHD will initially be calculated after the first six evaluable subjects accrued to each arm, and then will be calculated continuously.

l) In the event that an enrolled subject is not evaluable for the allo-engraftment end-point (for example, subject does not proceed to transplant, or subject dies of progressive malignancy or transplant-related toxicity within the first 100 days post-SCT), a replacement subject will be enrolled. It is anticipated that less than 10 of the enrolled subjects will be non-evaluable for the allo-engraftment end-point.

m) On arm IV of this protocol, the investigational Th2 cells are administered at day 14 post-HSCT. In the event that a subject enrolled to receive this cellular therapy suffers a serious adverse event during the first 14 days post-transplant, then a decision will be made to not administer Th2 cells to such a subject, as toxicity evaluation of the investigational cellular product will be significantly compromised; in the event that such a case were to occur, a replacement subject will be enrolled. Examples of serious adverse events that would trigger a decision to avoid Th2 cell infusion would be: severe engraftment syndrome or severe reduction in cardiac function (grade 4 severity). It is anticipated that less than 10% of subjects designated to receive Th2 cells will not be eligible to receive this therapy.

n) The purpose of Amendment N is to better estimate the rate of grade II to IV acute GVHD that associates with transplantation on arm IV. At the time of this amendment, the rate of grade II to IV acute GVHD on arm IV has been 12% (2/17); each of these cases were grade II on the basis of a stage 1 gut GVHD diagnosis. Accrual to arm IV will be increased from an initial value of 25 to a value of 40. With an accrual of 40 patients to arm IV, the maximum confidence interval width for the fraction of patients with grade II to IV acute GVHD will not exceed +/- 15%.

o) The purpose of amendment P is to initiate a new treatment arm, “Arm IVB”, that will evaluate the effect of Th2 cells that are manufactured using a short-term culture method (6-days of culture); these results will be compared to results of the previous arm IV (now termed “Arm IVA”) that evaluated the effect of Th2 cells manufactured using a longer-term culture method (12-days of culture). Twenty new patients will be transplanted on arm IVB. Thus, a total of 40 patients will be transplanted on arm IVA and 20 patients will be transplanted on arm IVB.

p) At this point in protocol implementation, the incidence of grade II to IV acute GVHD on arm IVA has been relatively low (3 cases out of 28; 10.7%). The upper 95% one-sided confidence interval for this observed incidence of acute GVHD is 25.4 %. As such, in arm IVB, the attainment of the new primary objective in terms of observing a low rate of acute GVHD in arm IVB similar to that observed in arm IVA will require that no more than 5 patients out of 20 (25%) transplanted on arm IVB will develop acute GVHD.

q) As such, with respect to the above-statement (p), accrual to arm IVB will be stopped at any point that a 6th patient on arm IVB develops acute GVHD.

r) It should be noted that the statistics for arm IVA are based on the first 28 patients transplanted out of a total of 40, and that the actual rate of acute GVHD in the total of 40 patients will be different from the current rate of 10.7%. As such, in the future, if this actual rate of acute GVHD on arm IVA is substantially different from the current observed rate (either substantially higher or lower), then a protocol amendment may be submitted that would modify the target for acute GVHD rate desired for arm IVB.

s) In terms of protocol accrual, accrual of patients to arms IVA and IVB will be sequential. That is, the entire cohort of 40 patients on arm IVA will be accrued, followed by the 20 patients to be accrued to arm IVB.

t) As a secondary objective evaluation, the actual percent chimerism per patient, primarily at day 28, will be compared between patients on arm IVA and IVB with a two-sample t-test or Wilcoxon rank sum test as appropriate.

u) The purpose of amendment R is to improve an ability to compare clinical results from arm IVA and arm IVB. At this point in protocol implementation, 40 patients have been transplanted on arm IVA. Twenty (20) patients were initially to be treated on arm IVB; 14 of these patients have been transplanted and the remaining 6 patients are either early in the protocol treatment or in the protocol screening process. In order to better compare arms IVA and IVB, a total of 40 patients will be accrued to each arm (that is, amendment R will allow for the transplantation of 20 additional patients on arm IVB).

v) The new primary objective relating to amendment R is to determine whether transplantation on arm IVB results in a higher frequency of patients with full donor lymphoid engraftment (compared to arm IVA). Full donor lymphoid engraftment is defined by $\geq$ 95% donor lymphoid chimerism at day 100 post-transplant. On arm IVA, 17/37 (46%) of evaluable transplant recipients had full donor lymphoid engraftment by this definition; by comparison, 4/5 (80%) of evaluable arm IVB patients have attained full donor lymphoid engraftment by this definition. With 40 evaluable patients in arm IVB (compared to 37 in arm IVA), there would be 82% power to detect a difference between 46% and 80% with full donor lymphoid chimerism using a two-sided 0.05 alpha level test.

w) A new secondary objective relating to amendment R will consist of an evaluation of the anti-tumor efficacy of arms IVA and IVB. At this point in protocol implementation for arm IVA, 6/20 (30%) of patients with high-risk malignancy have achieved a complete remission; high-risk malignancy is defined by a diagnosis of acute myelogenous leukemia, Hodgkin's Disease, or chemotherapy-refractory B cell malignancy. We project that approximately 20 of the intended 40 patients to be transplanted on arm IVB will have high-risk malignancy. If 20 patients in arm IVA were to be compared to 20 patients in arm IVB, there would be 82% power to detect a difference between a 30% complete response rate (in arm IVA) with a 70% complete response rate in arm IVB (using a one-sided 0.10 test).

x) A new stopping rule pertaining to acute GVHD on arm IVB will be instituted with amendment R; this stopping rule will take the place of the prior GVHD stopping rule that was defined in section "q" above. At this point in protocol implementation, acute GVHD grade II-IV has been observed in 5/14 patients; however, each patient has responded to cortico-steroid therapy. The new safety goal in arm IVB therapy will be to avoid a 10% rate of steroid-refractory acute GVHD. Accrual to arm IVB will stop if more than four patients out of the first 25 patients develop steroid-refractory acute GVHD. The statistical rationale for this stopping rule is as follows: the lower one-sided 80% confidence interval for 5/25 patients with steroid-refractory acute GVHD is 12.6%, which would exceed our goal of 10% or less.

y) With amendment U, a new primary objective has been added: to determine the rate of acute GVHD on a new arm IVC, which will evaluate Th2 cell therapy in the setting of a higher target level of sirolimus GVHD prophylaxis. Arm IVB is presently accruing patients, and is expected to have approximately 30% of patients experience grade II to IV acute GVHD. The goal of arm IVC is to determine if this rate can be lowered by increasing the sirolimus steady state drug level. If 40 patients are enrolled onto arm IVC, there would be 77% power to identify a difference between 30% acute GVHD on arm IVB and 10% acute GVHD on arm IVC, using a one-tailed 0.10 alpha level Fisher's exact test. Thus, enrolling 40 patients on

arm IVC would be associated with reasonable power to detect a difference of the magnitude identified using the test indicated.

z) The maximal protocol accrual ceiling prior to amendment U was 270 (135 recipients, 135 donors). Because 40 recipients and 40 donors will be added with amendment U, the new maximal protocol accrual ceiling will be 350 (175 recipients, 175 donors).

aa) The maximal protocol accrual ceiling prior to amendment Y was 350 (175 recipients, 175 donors). Because 15 recipients and 15 donors will be added with amendment Z to allow for 40 evaluable patients each in arms IVA, IVB, and IVC, the new maximal protocol accrual ceiling will be 380 (190 recipients, 190 donors).

bb) With Amendment AA, a new protocol objective will be to compare in a preliminary manner the transplant outcome of lymphoma patients receiving low-intensity allogeneic HCT using pre-emptive DLI with either manufactured donor T-Rapa cells (arm IVD, cohort 1) or unmanipulated donor T cells (arm IVD, cohort 2). Patients suitable for randomization (see section **1.7**) will be randomized to one of these treatment arms just prior to administration of the transplant preparative regimen.

cc) Ten patients will be accrued to each of these two new treatment arms. After accrual and sufficient post-transplant follow-up, we will informally compare the outcome of the two arms with respect to: conversion of mixed chimerism towards full donor chimerism, safety in terms of acute and late acute GVHD through day 180 post-transplant, and degree of malignant disease control.

dd) In an attempt to better characterize the two different types of DLI in terms of their ability to mediate anti-tumor effects, only patients with Hodgkin's or non-Hodgkin's lymphoma or chronic lymphocytic leukemia will be randomized.

ee) With only ten patients in each of the randomized arms, the study is not powered to detect in a statistically significant manner potential differences in clinical outcome (GVHD, engraftment, or anti-tumor). Rather, it is more likely that these pilot data will be critical for the design of subsequent, more definitive clinical trials; specifically, it will be important to establish that administration of unmanipulated donor T cells on cohort 2 represents a safe and potentially efficacious therapy in the context of the unique, low-intensity transplant regimen that we have developed on this protocol 04-C-0055.

ff) Patients will be initially registered with Central Registration at the time donor and recipient sign the consents. In most cases, patients will initially not be randomized to cohort 1 vs. cohort 2; instead, patients will proceed to pre-transplant chemotherapy in an attempt to reduce the patient CD4 count to < 200 and to achieve malignant disease control before transplantation. Once such patients are deemed ready for transplantation, they will be randomized to either cohort 1 or cohort 2; randomization will occur through the Harris contractor under the supervision of Dr. Steinberg (block randomization will be used). In some cases, at the time of initial enrollment, a patient may be ready for transplant (CD4 count is < 200 and disease is under adequate control); in such cases, the patient will be randomized and proceed directly to transplantation.

gg) Randomization will be stratified according to patients deemed to be "high-risk" or "standard risk", according to the PI and Lead AI joint assessment at the time of randomization. Patients with chemotherapy-refractory disease and patients with Kahl Category III histology will be considered "high-risk".

hh) If an initially enrolled lymphoma subject is determined to be too young (< 18 year of age) or too high of a risk for randomization during the pre-transplant treatment phase (for example,

due to disease progression), the patient will not be randomized. With this provision, we will have a greater chance that each randomized and transplanted patient is evaluable on the protocol-defined therapy at the day 100 and day 180 post-transplant time points. As an alternative transplant option, such patients may be treated on arm IVD, cohort 3, which will evaluate transplantation without the Flu/Cy preparative regimen and with multiple infusions of T-Rapa cells.

ii) If a randomized patient develops a toxicity prior to the day 14 post-transplant DLI therapy that precludes DLI administration (such as engraftment syndrome), then the patient will be removed from the randomization scheme, will not receive a day 14 DLI, and will then be managed by standard transplant practices. A replacement patient will be accrued. Alternatively, for patients with resolving toxicity and mixed chimerism or persistent tumor, the patient may stay on study, with a delay in the pre-emptive DLI permitted until day 35 post-transplant.

jj) A maximum of 10 patients will also be transplanted on arm IVD, cohorts 1 and 2.

kk) For cohorts 1 and 2, stopping rules will be utilized to help ensure safety. Specifically, if at any point during protocol implementation: (a) if more than 1 of the first 10 patients develop steroid-refractory acute GVHD in the first 100 days post-transplant, then such therapy will be halted pending further Branch and IRB review; and (b) if more than 1 of the first 10 patients develop graft rejection in the first 100 days post-transplant, then such therapy will be halted pending further Branch and IRB review.

ll) A new statistical objective has been instituted for amendment CC: cohort 3 will be expanded from 10 patients to 40 patients in order to provide sufficient patients to permit a potentially definitive comparison with prior cohorts with respect to GVHD. Cohorts 4B and 4C taken together had 35/86 patients (41%) experience grade II-IV aGVHD. It would be of interest to determine if the strategy evaluated in cohort 3 would be associated with a much smaller rate of grade II-IV aGVHD than the combination of cohorts 4B and 4C. With 40 evaluable patients on cohort 3, there would be 81% power detect a difference between 15% with grade II-IV aGVHD on cohort 3 vs. 41% on 4B and 4C with 86 patients combined, using a two-tailed 0.05 significance level with a Fisher's exact test.

mm) To help ensure patient safety during expanded accrual to cohort 3, the following stopping rules will be instituted: (1) Stopping rule for severe acute GVHD. In the combined comparison cohorts 4B and 4C, the rate of severe acute GVHD (defined as grades III to IV through day 100 post-transplant) was 11/86 (12.8%). Therefore, further protocol accrual to cohort 3 will stop if the rate of severe acute GVHD: exceeds 3 cases out of the first 10 evaluable patients; exceeds 5 cases out of the first 20 evaluable patients; or exceeds 6 cases out of the first 30 patients. In each case, the lower one-sided 90% confidence interval bound would exceed 12.8% and thus could be considered to be inconsistent with 12.8% and worthy of stopping on that basis (4/10 has a lower one sided 90% CI bound of 18.8%; 6/20 has a lower bound of 16.6%, and 7/30 has a lower bound of 13.5%). (2) Stopping rule for deficiency of GVL effect. In the combined comparison cohorts 4B and 4C, the rate of death due primarily to progressive disease in the first 180 days post-transplant was ten cases out of 86 patients (11.6%). Therefore, further protocol accrual to cohort 3 will stop if the rate of death due to progressive disease in the first 180 days post-transplant: exceeds 3 cases out of the first 10 evaluable patients; exceeds 4 cases out of the first 20 evaluable patients; or exceeds 6 cases out of the first 30 patients. In each case, the lower one-sided 90% confidence interval bound would exceed 11.6% and thus could be considered to be inconsistent with

11.6 % and worthy of stopping on that basis (4/10 has a lower one sided 90% CI bound of 18.8%; 5/20 has a lower bound of 12.7%, and 7/30 has a lower bound of 13.5%).

nn) The maximal protocol accrual ceiling prior to amendment CC was 450 (225 recipients, 225 donors). Up to 30 recipients and 30 donors will be added with amendment CC to allow for accrual of 40 patients to cohort 3; as such, the new maximal protocol accrual ceiling will be 510 (255 recipients, 255 donors).

8 COLLABORATIVE AGREEMENTS

8.1 AGREEMENT TYPE

The coded serum samples and Human Peripheral blood Mononuclear Cells will be sent to the Scripps Research Institute as a part of this protocol using a Material Transfer Agreement (MTA) between the Scripps Research Institute and the NCI.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

Patients with lymphoid malignancy and leukemia will be the subjects for this study. These patient groups were chosen because allogeneic bone marrow transplantation represents a potentially curative treatment approach for these diseases. Leukemia and lymphoma affect all races and genders. In general, the age group of selected patients will be those individuals over age 40; because myeloablative preparative regimens are associated with a high degree of morbidity and mortality in this age group, such individuals are most likely to benefit from the reduced-intensity transplant approach used in this study. Individuals with HIV disease will not be candidates for this protocol, due to the high rate of post-transplant complications in this group. Individuals who are pregnant or lactating will not be candidates for this protocol, due to risk to the fetus or newborn.

9.2 PARTICIPATION OF CHILDREN

Children ages 16 years and older will be evaluated as recipients on this protocol. Evaluation of this transplant strategy in children younger than 16 would require a separate protocol. Donors below the age of 11 will not be included on this protocol since the protocol involves greater than minimal risk without prospect of direct benefit to individual patients for the donor population. In the rare circumstance that a suitable adult donor is not available, a minor donor will be used.

Physicians, nurses, and multidisciplinary support teams of the POB, NCI, and CC will provide patient care for recipients ages 16-17 the donors ages 11-17. The staff of the POB has expertise in the management of children with complex oncologic disorders and complications of therapy. Full pediatric support and subspecialty services are available at the NIH CC.

9.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **9.4**), all NIH Clinical Center recipients subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become

incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.
Donor subjects who become incapacitated or cognitively impaired during the course of the study will come off study since there are no direct benefit to the donor subjects.

9.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients may obtain direct benefit from anti-tumor activity of the induction chemotherapy and pre-transplant chemotherapy. However, for the patient groups to be enrolled on this study, the chemotherapy administered would not represent a curative modality; rather, the intent of the chemotherapy administration is to provide short-term disease remission and to suppress the immune system prior to the allogeneic transplantation. The chemotherapy utilized in this study has the risk of direct drug toxicity; in addition, immune suppression resulting from chemotherapy administration is associated with an increased risk of opportunistic infections. However, it is anticipated that these risks will be lower than the risks associated with traditional allogeneic transplantation utilizing total body irradiation.

The primary risk associated with allogeneic stem cell transplantation is graft-versus-host disease (GVHD), which remains the primary cause of morbidity and mortality post-transplant. Each patient on this study will receive standard cyclosporine GVHD prophylaxis, plus an additional immune intervention that may further reduce the chance of developing GVHD; at the time of this current protocol amendment E, this immune intervention consists of cyclosporine plus sirolimus therapy through day 14 post-transplant with the further intervention of donor Th2 cell therapy on either day 14 post-SCT (arm IV) or day 0 of transplant (arm V). These additional immune interventions, however, are not proven, and as such, it is not known whether study recipients will be protected against GVHD to a greater extent than recipients of cyclosporine alone.

9.5 DISCUSS WHY THE RISKS TO SUBJECTS ARE REASONABLE IN RELATION TO THE ANTICIPATED BENEFITS AND IN RELATION TO THE IMPORTANCE OF THE KNOWLEDGE THAT MAY REASONABLY BE EXPECTED TO RESULT

Patients who are recipients on this study may be directly benefited by this treatment protocol. The chemotherapy administered may provide some form of remission from their malignancy. Successful allogeneic stem cell transplantation may offer patients a curative therapy for their malignancy. It is hypothesized that the transplant approach used in this study will result in the successful transfer of allogeneic stem cells with reduced morbidity and mortality from the transplant preparative regimen and with reduced graft-versus-host disease. It is also anticipated that this study will provide scientific information relevant to attempts to prevent graft rejection, to limit GVHD, to mediate graft-versus-leukemia or graft-versus-tumor effects, to reduce transplant-related mortality, and to extend the application of allogeneic transplantation to those patients who lack an HLA-matched donor.

9.6 CONSENT AND ASSENT PROCESSES AND DOCUMENTS

a) The procedures and treatments involved in this protocol, with their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained

to the recipient. Similarly, the procedures and treatments involved in this protocol, with their attendant risks and discomforts, will be carefully explained to the donor. A signed, informed consent document will be obtained from both the recipient and the donor, or if he/she is < 18 years of age, the subject's parents/guardian.

b) The original signed informed consent documents will be kept with the patient's chart. The Data Management Section will also retain a copy of the informed consent document. A copy of the informed consent documents will also be given to the recipient and/or donor patient's parents/guardian if applicable.

c) The Clinical Coordinator, Data Management Section, will ascertain the date of IRB approval before registering the first patient.

- Informed consent will be obtained from all donors and recipients entered on this trial or the subject's parents/guardian (if he/she is < 18 years of age). The procedures and treatments involved in this protocol, with their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the recipient and/or parent/guardian. Where deemed appropriate by the clinician and the child's parent(s) or guardian, the child will also be included in all discussions about the trial and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. Verbal assent will be obtained as appropriate for children ages (12 years and older) and the parent or guardian will sign the designated line on the informed consent attesting to the fact that the child has given assent. Children under the age of 12 years will not be required to provide assent as they typically do not have the cognitive ability to fully understand the nature of research. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide verbal assent. All children will be contacted after they have reached the age of 18 to determine whether they wish to continue on the trial and informed consent will be obtained from them at that time.

The investigators are requesting a waiver from the IRB to allow only one parent to sign the informed consent to enter a child on the protocol. As many patients must travel to the NIH from long distances at substantial expense, requiring both parents to be present for the consent process could be a financial hardship for many families. In addition, many minors in the United States have only 1 parental guardian for medical decisions. Thus, this study requests the acceptance of the signature of either: 1) the sole primary decision maker for the patient, or 2) a single signature representing the consent of both parties. . In situations where there is joint custody of a child, both parents must sign consent. If only one parent can be present at NIH, the other parent's consent can be obtained by telephone via the procedure described in section **9.6.1** The investigational nature and research objectives of this trial, the attendant risks and benefits associated with the procedures and treatments of this trial, and the potential for alternative therapies will be carefully explained in layman's terms for patients and guardians. A signed informed consent document will be obtained by patient or legal guardian prior to entry onto the study by the Principal Investigator or/and Associate Investigator at each participating institution. Each participating institution will be responsible for its own informed consent form, and will obtain IRB approval of the informed consent initially at their institution and then at the NCI IRB.

d) Consent for the optional biopsy will be obtained at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

9.6.1 Telephone consent and re-consent procedure

The following procedure may be used in cases of re-consent or for initial consent in cases where the consent of both parents of a minor participant is required as discussed in section 9.6the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent. The original informed consent document will be,sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

9.6.2 Informed consent of non-English speaking subjects:

We anticipate the enrollment of Spanish 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.

9.6.3 Short form consent process for non-English speaking patients

If there is an unexpected enrollment of a research participant for whom 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, OSHRP SOP 12, 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.
Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation. Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).
We request prospective IRB approval of the use of the short form process and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

9.7 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

Stored samples include peripheral blood cells and/or bone marrow cells from the transplant donors and from the transplant recipients both before and after the transplant. These samples are stored under liquid nitrogen cryopreservation in research freezers maintained in the laboratory of the study PI, Dr. Pavletic, and in the laboratory of research associate investigator, Dr. Fran Hakim. The research purpose of these samples is to perform laboratory experiments relating to the scientific objectives of this protocol, specifically: to understand the role of Th1/Th2 cytokine biology in the modulation of human transplant responses, including graft-versus-host disease, graft rejection, and graft-versus-tumor effects; and to understand the biology of immune reconstitution that occurs after allogeneic hematopoietic cell transplantation. The cells are stored

in a coded manner, with only the PI or the AI able to track specific samples to the identity of the donor or recipient. The samples will be stored until the completion of the protocol; at that time, the samples will be destroyed. The PI will report to the IRB any loss or destruction of samples, or any new use of the collected samples.

10 MULTI-INSTITUTIONAL GUIDELINES

10.1 IRB APPROVALS

a) The Coordinating PI (NCI PI) will ensure that no patient is entered onto the trial at a participating institution without full IRB approval of the study. Thus, the NCI IRB must approve the addition of each participating institution to the protocol; furthermore, a copy of the local IRB approval from each participating institution will be required before NCI IRB approval is granted.
b) The PI will provide the NCI IRB with a copy of the participating institution's approved annual continuing review.

10.2 AMENDMENTS AND CONSENTS

All amendments to the protocol or the NCI consent are to be approved by the NCI IRB and then submitted to the participating institution's IRB for approval. The approval memos need to be sent back to the NCI PI.

10.3 DATA COLLECTING AND TOXICITY REPORTING

Guidelines for adverse event reporting are detailed below in section **10**. All SAEs from each participating institution are reported simultaneously to the participating institution IRB (according to the local IRB guidelines) and to the NCI PI. The event may be reviewed in a conference call between PIs if deemed necessary by the NCI PI prior to submission of the SAE to the NCI IRB. The Sponsor will make the decision whether the SAE should be reported to the FDA IND file in an expedited manner or through inclusion in the IND annual report. The IND sponsor remains responsible for FDA reporting for the entire study.

10.4 FDA AUDIT

The investigator may be subject to a field audit by FDA inspectors to validate the participation of study subjects, adherence to the study protocol, and to verify the data reported in NCI C3D. This audit could occur while the study is in progress or several years after the study is completed. All of the patients' records and other study documentation must be filed and accessible on short notice (within 3 to 5 days) during the study and subsequent retention period. The NCI PI should be notified immediately if the participating center has been contacted by the FDA for an audit. In addition, quality assurance audits may be performed by NCI contractor. at various points throughout the study.

11 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

11.1 DEFINITIONS

11.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event

is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted in Appendix D.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **11.2** and **11.3.**

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

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

11.1.2 Suspected adverse reaction

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

11.1.3 Unexpected adverse reaction

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

11.1.4 Serious

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

11.1.5 Serious Adverse Event

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

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

11.1.6 Disability

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

11.1.7 Life-threatening adverse drug experience

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

11.1.8 Protocol Deviation (NIH Definition)

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

11.1.9 Non-compliance (NIH Definition)

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

11.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

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

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

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

11.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

11.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems, and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease

- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

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

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

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

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

11.2.3 NCI-IRB Reporting of IND Safety Reports

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

11.3 IND SPONSOR REPORTING CRITERIA

An investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

- All Grade 5 (fatal) events (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- All other serious adverse events including deaths due to progressive disease must be reported within one business day

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to the IND sponsor at : brian@rapatherapeutics.com

11.3.1 Reporting Pregnancy

11.3.1.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".
Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as **Grade 3** "***Pregnancy, puerperium and perinatal conditions - Other (pregnancy)***" under the ***Pregnancy, puerperium and perinatal conditions*** SOC.
Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.
If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.
The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.
The same timelines apply when outcome information is available.

11.3.1.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study experimental procedure, and for one year after transplant.
Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until (360 days*)* after the last dose should, if possible, be followed up and documented.

11.4 DATA AND SAFETY MONITORING PLAN

11.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

11.4.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

11.4.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

11.5 STOPPING RULES

a) For arm IVD, cohorts 1 and 2, stopping rules will be utilized to help ensure safety. Specifically, if at any point during protocol implementation: (a) if more than 1 of the first 10 patients develop steroid-refractory acute GVHD in the first 100 days post-transplant, then such therapy will be halted pending further Branch and IRB review; and (b) if more than 1 of the first 10 patients develop graft rejection in the first 100 days post-transplant, then such therapy will be halted pending further Branch and IRB review.

b) For arm IVD, cohort 3, the following stopping rules will be utilized: (1) Stopping rule for severe acute GVHD. In the combined comparison cohorts 4B and 4C, the rate of severe acute GVHD (defined as grades III to IV through day 100 post-transplant) was 11/86 (12.8%). Therefore, further protocol accrual to cohort 3 will stop if the rate of severe acute GVHD: exceeds 3 cases out of the first 10 evaluable patients; exceeds 5 cases out of the first 20 evaluable patients; or exceeds 6 cases out of the first 30 patients. In each case, the lower one-sided 90% confidence interval bound would exceed 12.8% and thus could be considered to be inconsistent with 12.8% and worthy of stopping on that basis (4/10 has a lower one sided 90% CI bound of 18.8%; 6/20 has a lower bound of 16.6%, and 7/30 has a lower bound of 13.5%). (2) Stopping rule for deficiency of GVL effect. In the combined comparison cohorts 4B and 4C, the rate of death due primarily to progressive disease in the first 180 days post-transplant was ten cases out of 86 patients (11.6%). Therefore, further protocol accrual to cohort 3 will stop if the rate of death due

to progressive disease in the first 180 days post-transplant: exceeds 3 cases out of the first 10 evaluable patients; exceeds 4 cases out of the first 20 evaluable patients; or exceeds 6 cases out of the first 30 patients. In each case, the lower one-sided 90% confidence interval bound would exceed 11.6% and thus could be considered to be inconsistent with 11.6 % and worthy of stopping on that basis (4/10 has a lower one sided 90% CI bound of 18.8%; 5/20 has a lower bound of 12.7%, and 7/30 has a lower bound of 13.5%).

c) Should any concerns regarding the safety of this protocol arise, then the IRB, CTEP, FDA and all investigators whose IND has been cross-referenced on this protocol will be notified of any such concern.

11.6 RECORD KEEPING

a) All patients must have signed an Informed Consent and an on-study confirmation of eligibility form filled out before entering on the study.
b) A summary of protocol data, generated from the C_3D database, will be submitted every six months to the NCI IRB for continuing review, and annually to the FDA.
c) Data will be also sent to the International BMT Registry (IBMTR).
d) Within 2 months of study completion, a summary will be sent to IDB/CTEP. A status report will be submitted and may be presented at meetings as requested.
e) Complete records must be maintained on each patient; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on the C_3D database from which formal analyses are done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flowsheets, specialty forms for pathology, radiation, or surgery; adverse event assessment; and off-study summary sheet, including a final assessment by the treating physician.

12 PHARMACEUTICAL INFORMATION

12.1 CYCLOPHOSPHAMIDE (CTX, CYTOXAN, NSC-26271)

12.1.1 Supply: Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.

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

12.1.3 Storage and Stability: The vials are stored at room temperature. Following reconstitution as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8° C.

12.1.4 Route of Administration: The cyclophosphamide used in this regimen will be mixed in 100 ml 0.9% NaCl and given as an IVPB over 30 minutes in the induction regimen (EPOCH-F/R). In the preparative regimen, it is given over two hours.

12.1.5 Toxicities: 1) Nausea and vomiting-symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam]. 2) Water retention- may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hrs after IV administration, necessitating frequent accurate

assessment [q 1-2 hrs] of intake, urine output and urine specific gravity. This effect can be counteracted by furosemide. Fluid restriction is not feasible during administration of high dose cyclophosphamide. 3) Cardiomyopathy- may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed in patients receiving the dose of cyclophosphamide used in this protocol. In an attempt to minimize this complication, patients with significant cardiac dysfunction are excluded from this protocol [see patient eligibility]. Congestive failure is managed according to standard medical therapeutics. 4) Hemorrhagic cystitis- this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites. Although subclinical hematuria is not uncommon at this dose level, clinically significant hematuria or serious hemorrhage can usually be avoided by maintaining a high urine volume and frequent voidings. Diuresis is maintained for 24 hrs after completion of last dose by parenteral infusions of 0.9% NaCl with potassium chloride. Careful monitoring of serum and urine electrolytes is mandated. Furosemide may be required to ensure this diuresis. Continuous bladder irrigation may be used for control of significant hematuria. 5) Sterility. 6) Less commonly, other serious complications such as pulmonary fibrosis and secondary malignancy may occur. 7) Less common but reversible toxicities include alopecia and skin rash.

12.2 FILGRASTIM (G-CSF, NEUPOGEN®)

12.2.1 Supply: Commercially available as filgrastim injection in a concentration of 300μg/ml in 1ml (300μg) and 1.6ml (480μg) vials.

12.2.2 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 to a concentration greater than 5μg/ml. Dilution of filgrastim to a final concentration of less than 5μg/ml is not recommended at any time. Do not dilute filgrastim at any time; product may precipitate. Filgrastim diluted to concentrations between 5 and 15μg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/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. The dose may be "rounded down" to within 10% of patient's calculated dose to use the drug cost-effectively.

12.2.3 Storage and Stability: Filgrastim for injection should be stored in the refrigerator at 2º to 8ºC (36º to 46ºF). Avoid shaking.

12.2.4 Administration: Subcutaneous injection is preferred. If clinically indicated, filgrastim may be administered as an intravenous infusion over 4 or 24 hours.

12.2.5 Toxicities:1) Medullary bone or skeletal pain is the most commonly reported toxicity. 2) In addition, reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase are common laboratory abnormalities. 3) Four cases of splenic rupture have

been reported in healthy donors when given filgrastim or other myeloid growth factors for peripheral blood stem cell mobilization; 1 of these cases resulted in fatality. Five additional cases of splenic rupture have been reported in cancer patients undergoing chemotherapy or peripheral blood stem cell mobilization; splenic rupture may have contributed to deaths in 2 of these cases. One additional death due to splenic rupture after filgrastim therapy was reported to the manufacturer without additional information. According to the manufacturer, the reporting rate for splenic rupture with filgrastim is less than 1 in 486,000.

12.3 CYCLOSPORINE (GENGRAF, SANDIMMUNE, NEORAL)

12.3.1 Supply: 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.

12.3.2 Preparation: For parenteral doses, each milliliter of concentrate (50mg/ml) should be diluted in 20 to 100ml 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 (see Storage and Stability, section **9.7**). Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.

12.3.3 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 2mg/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. D) Administration – Cyclosporine may be given intravenously over 2 hours or orally.

12.3.4 Toxicities: 1) Acute cyclosporine nephrotoxicity is usually manifested by a moderate decline in renal excretory function, which is readily reversible by a decrease in drug dosage. Although some degree of transient renal dysfunction may occur in patients with therapeutic levels of cyclosporine, significant renal toxicity is associated with elevated trough levels. 2) In addition to an increase in BUN and creatinine, hyperkalemic hyperchloremic acidosis, low fractional excretion of sodium and the onset of hypertension with hypomagnesemia are seen with cyclosporine nephrotoxicity. 3) Hypertension occurs in up to 60% of patients. 4) Hypomagnesemia, which may contribute to cyclosprorine neurotoxicity in the form of seizures, cerebellar ataxia, depression, and coma. 5) Dose-related hepatotoxicity, manifested by elevation of serum transaminases and bilirubin, has been reported.

12.4 SIROLIMUS (RAPAMYCIN) (RAPAMUNE®, WYETH-AYERST LABORATORIES)

12.4.1 Supply: For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. For in vitro Th2 cell expansion,

sterile oral solution will be purchased by the NIH Clinical Center Department of Transfusion Medicine, Cell Processing Section.

12.4.2 Storage and Stability: Oral tablets should be stored 20-25 C° (68-77 F°). Oral solution should be refrigerated (2-8 C° or 36-46 F°); to help ensure sterility of the oral solution, one sterile bottle of sirolimus will be purchased for each donor Th2 cell culture for media supplementation for that particular culture.

12.4.3 Administration: Oral tablets should be administered approximately 4 hours after cyclosporine administration. Because food, in particular fatty foods, can decrease the absorption of sirolimus, the tablets should be administered between meals (lunch and dinner).

12.4.4 Toxicities: 1) Sirolimus induces immune suppression, which has been associated with opportunistic infection and an increased rate of malignancy, particularly skin cancer. 2) Some individuals may develop hypersensitivity to sirolimus. 3) May cause an increase in cholesterol and triglycerides, which may be associated with pancreatitis. 4) With long-term administration, may result in impaired renal function. 4) Metabolism is via the cytochrome p450 pathway, and as such, co-administration of voriconazole is prohibited on this protocol (for first two weeks post-transplant), as this combination will result in prolonged blood levels of sirolimus.

12.5 FLUDARABINE (FLUDARA, BERLEX LABORATORIES)

12.5.1 Supply: Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludara is stored at room temperature.

12.5.2 Preparation: FLUDARA IV should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7–8.5. Fludarabine will be diluted in 100 to 125ml of either 5% dextrose in water or 0.9% sodium chloride, and infused IV over 30 minutes.

12.5.3 Storage and Stability: Reconstituted FLUDARA IV is chemically and physically stable for 24 hours at room temperature or for 48 hours if refrigerated. Because reconstituted FLUDARA IV contains no antimicrobial preservative, care must be taken to assure the sterility of the prepared solution; for this reason, reconstituted FLUDARA IV should be used or discarded within 8 hours.

12.5.4 Administration: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

12.5.5 Toxicities include myelosuppression (dose limiting), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, auto-immune hemolytic anemia, auto-immune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (intersititial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate.

Such toxicity has only rarely been demonstrated at the 25-30 mg/m^2/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agents.

12.6 ETOPOSIDE, DOXORUBICIN, AND VINCRISTINE

For infusional chemotherapy, etoposide, doxorubicin, and vincristine will be combined in a single ('3-in-1') admixture, diluted in a volume of 0.9% Sodium Chloride (NS), Injection, USP, that is based on the amount of etoposide needed to complete one day of treatment:

Etoposide Dose	Volume of Fluid Containing a Daily Dose	Volume of Overfill (fluid + drug)	Volume To Infuse (over 24 hours)	Administration Rate
≤130 mg	528 mL	25 mL	528 mL	22 mL/hour
≥131 mg	1056 mL	25 mL	1056 mL	44 mL/hour

All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine *PLUS* 25 mL overfill (excess) fluid and a proportional amount of drug to compensate for volume lost in administration set tubing.
Before dispensing 3-in-1 admixtures, Pharmacy staff will [1] purge all air from the drug product container, [2] attach an administration set appropriate for use with a portable pump and the set will be [3] primed close to its distal tip and [4] capped with a Luer-locking cap. Bags will be exchanged daily for 4 consecutive days to complete a 96-hour drug infusion. Portable pumps used to administer etoposide + doxorubicin + vincristine admixtures will be programmed to deliver one of two fixed volumes at one of two corresponding fixed rates based on the amount of etoposide and fluid that is ordered (see table, above). At the end of an infusion, some residual fluid is expected because overfill fluid and drug were added; however, nurses are asked to return to the Pharmacy for measurement any drug containers that appear to contain a greater amount of residual drug than expected.
Etoposide, doxorubicin, and vincristine will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Studies conducted by the Pharmaceutical Development Service, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride for Injection (at concentrations, respectively, of 1, 25, and 125 mcg/ml; 2, 50, and 250 mcg/ml; and 2.8, 70, and 350 mcg/ml) are stable for at least 48 hours at room temperature when protected from light. Etoposide toxicities include nausea, vomiting, stomatitis, diarrhea, neutropenia, thrombocytopenia, and alopecia. Secondary AML has been associated with etoposide. Bradycardia and hypotension are sometimes observed with etoposide administration. Doxorubicin toxicities include myelosuppression, and cardiotoxicity (cardiotoxicity is particularly noted after cumulative doses of greater than 550 mg/m^2). Other doxorubicin toxicities include nausea, vomiting, stomatitis, diarrhea, alopecia, and tissue necrosis (in event of skin infiltration). Vincristine toxicities are primarily neurologic and include paresthesias, jaw pain, ataxia, foot-drop, cranial nerve palsies,

paralytic ileus, constipation, abdominal pain, and loss of deep tendon reflexes. Vincristine is also a vesicant, and occasionally causes alopecia and myelosuppression.

12.7 PREDNISONE

12.7.1 Supply: Prednisone will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Prednisone is commercially available as tablets, in strengths of 1, 2.5, 5, 20, and 50 mg.

12.7.2 Storage and Stability: Prednisone tablets should be stored in the container provided away from heat. The product labeling bears the manufacturer's expiration dating for stability.

12.7.3 Administration: Prednisone will be administered at a dose of 60 mg/m^2 orally on days 1, 2, 3, 4, and 5 of the induction chemotherapy regimen. In patients unable to tolerate oral medication, methylprednisolone can be substituted at an equivalent dosage, diluted in a small volume (e.g. 25-50ml) of 5% dextrose in water or 0.9% sodium chloride and infused over 15 minutes. To reduce gastrointestinal side effects, prednisone should be taken with food.

12.7.4 Toxicities: Prednisone frequently causes gastritis, immunosuppression, muscle wasting, fluid retention, and hyperglycemia.

12.8 RITUXIMAB (RITUXAN)

12.8.1 Supply: Rituximab will be obtained commercially and is supplied as a 10 mg/ml sterile, preservative-free solution for injection, in vials of 100 mg and 500 mg.

12.8.2 Preparation: The desired dose will be diluted to a final concentration of 1 to 4 mg/mL in either 0.9% Sodium Chloride or D5W. c) Storage and Stability – Rituximab vials should be stored at 2–8°C (36–46°F) and should be protected from direct sunlight.

12.8.3 Administration: For first infusion, the rituximab solution for infusion should be administered intravenously at an initial rate of 50 mg/hr. Rituximab should not be mixed or diluted with other drugs. If hypersensitivity or infusion reactions do not occur, escalate the infusion rate in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If hypersensitivity (non-IgE-mediated) or an infusion reaction develops, the infusion should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of patient symptoms. If the first infusion is tolerated well, infusions during subsequent cycles of EPOCH-F/R can begin at a rate of 100 mg/hr and be increased by 100 mg/hr increments at 30-minute intervals. However, if the first infusion is not tolerated well, then the guidelines for the initial infusion should be followed for subsequent administration. Premedication will routinely be administered 30 to 60 minutes prior to the beginning of each rituximab infusion, consisting of acetaminophen 650 mg PO and diphenhydramine 50 mg IV.

12.8.4 Toxicities: a) The most serious adverse reactions caused by rituximab include infusion reactions, tumor lysis syndrome, mucocutaneous reactions, hypersensitivity reactions, cardiac arrhythmias and angina, and renal failure. B) Fatal and Severe Infusion Reactions: Deaths within 24 hours of rituximab infusion have been reported. Approximately 80% of fatal infusion reactions occurred in association with the first infusion. Severe reactions typically occurred during the first infusion with time to onset of 30 to 120 minutes. Signs and symptoms of severe infusion reactions may include

hypotension, angioedema, hypoxia or bronchospasm, and may require interruption of rituximab administration. The most severe manifestations and sequelae include pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. In the reported cases, the following factors were more frequently associated with fatal outcomes: female gender, pulmonary infiltrates, and chronic lymphocytic leukemia or mantle cell lymphoma. *Management of severe infusion reactions:* The rituximab infusion should be interrupted for severe reactions and supportive care measures instituted as medically indicated (e.g., intravenous fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen). In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Patients requiring close monitoring during first and all subsequent infusions include those with pre-existing cardiac and pulmonary conditions, those with prior clinically significant cardiopulmonary adverse events and those with high numbers of circulating malignant cells (= 25,000/mm^3) with or without evidence of high tumor burden. C) Tumor Lysis Syndrome (TLS): Acute renal failure requiring dialysis with instances of fatal outcome has been reported in the setting of TLS following treatment with rituximab. Rapid reduction in tumor volume followed by acute renal failure, hyperkalemia, hypocalcemia, hyperuricemia, or hyperphosphatasemia, have been reported within 12 to 24 hours after the first rituximab infusion. Rare instances of fatal outcome have been reported in the setting of TLS following treatment with rituximab. The risks of TLS appear to be greater in patients with high numbers of circulating malignant cells (= 25,000/mm^3) or high tumor burden. Prophylaxis for TLS should be considered for patients at high risk. Correction of electrolyte abnormalities, monitoring of renal function and fluid balance, and administration of supportive care, including dialysis, should be initiated as indicated. Following complete resolution of the complications of TLS, rituximab has been tolerated when re-administered in conjunction with prophylactic therapy for TLS in a limited number of cases. D) Severe Mucocutaneous Reactions: Mucocutaneous reactions, some with fatal outcome, have been reported in patients treated with rituximab. These reports include paraneoplastic pemphigus (an uncommon disorder which is a manifestation of the patient's underlying malignancy), Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. The onset of the reaction in the reported cases has varied from 1 to 13 weeks following rituximab exposure. Patients experiencing a severe mucocutaneous reaction should not receive any further infusions and seek prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and guide subsequent treatment. The safety of readministration of RITUXAN to patients with any of these mucocutaneous reactions has not been determined. E) Infusion reactions and lymphopenia are the most commonly occurring adverse reactions. Mild to moderate infusion reactions consisting of fever and chills/rigors occur in the majority of patients during the first rituximab infusion. Other frequent infusion reaction symptoms included nausea, pruritus, angioedema, asthenia, hypotension, headache, bronchospasm, throat irritation, rhinitis, urticaria, rash, vomiting, myalgia, dizziness, and hypertension. These reactions generally occur within 30 to 120 minutes of beginning the first infusion, and resolved with slowing or interruption of the rituximab infusion and with supportive care (diphenhydramine, acetaminophen, IV 0.9% NaCl, and vasopressors). In an analysis of data from 356 patients with relapsed or

refractory, low-grade NHL who received 4 (N = 319) or 8 (N = 37) weekly infusions of rituximab, the incidence of infusion reactions was highest during the first infusion (77%) and decreased with each subsequent infusion (30% with fourth infusion and 14% with eighth infusion).

12.9 DIPHENHYDRAMINE

12.9.1 Supply: Commercially available. Diphenhydramine HCl injection is available in an injectable solution at a 50mg/ml concentration in single dose ampules, syringes and vials as well as multi-dose vials from multiple manufacturers.

12.9.2 Preparation: Diphenhydramine HCl may be given by direct intravenous injection without additional dilution. Alternatively the prescribed dose may be diluted in a small volume (e.g. 25-50ml) of 5% dextrose in water (D5W) or 0.9% sodium chloride (NS) and infused over 10-15 minutes. C) Storage and Stability: Store commercially available injectable product at controlled room temperature.

12.9.3 Administration – Diphenhydramine HCl injection may be administered by direct IV injection (IV push) at a rate generally not exceeding 25mg/min. Alternatively, diphenhydramine HCl injection may be diluted and given over 10-15 minutes (see Preparation). E) Toxicities include sedation, sleepiness, dizziness, disturbed coordination, epigastric distress, thickening of bronchial secretions. Diphenhydramine can provide additive effects with alcohol or other CNS depressants. Diphenhydramine can cause anticholinergic side effects (e.g. dry mouth, fixed or dilated pupils, flushing, urinary retention). Diphenhydramine should be used with caution in patients with a history of bronchial asthma, increased intraocular pressure, hyperthyroidism, cardiovascular disease or hypertension.

12.10 ACETAMINOPHEN

12.10.1 Supply: Commercially available as 325 mg or 500 mg tablets for oral administration from multiple manufacturers. B) Storage: Store at controlled room temperature. C) Administration: Oral. For analgesia and antipyresis, the usual dose is 650 to 1000 milligrams every 4 to 6 hours, to a maximum of 4 grams/day. D) Toxicities: No toxicities are anticipated to result from single doses of acetaminophen administered as premedication for rituximab infusions.

12.11 VALACYCLOVIR (VALTREX®)

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

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

12.11.3 Storage and Stability: Oral tablets should be stored at 15º to 25ºC (59º to 77ºF). d) Administration: Oral. E) Toxicities: 1) Nausea and/or vomiting, headache, dizziness, abdominal pain, dysmenorrhea, arthralgia, acute hypersensitivity reactions, elevations in liver enzyme laboratory values (e.g. AST). 2) Renal failure and CNS symptoms have

been reported in patients with renal impairment who received valacyclovir or acyclovir at greater than the recommended dose. Dose reduction is recommended in this patient population (refer to the manufacturer's labeling for dose adjustment guidelines).

12.12 FLUCONAZOLE (DIFLUCAN®)

12.12.1 Supply: Commercially available as 50 mg, 100 mg, 150 mg and 200 mg tablets, or as a powder for oral suspension for reconstitution at a concentration of 10mg/ml or 40 mg/ml. Parenteral fluconazole is available in a solution for injection at a concentration of 2 mg/ml in glass bottles and Viaflex® Plus plastic containers containing either 100ml (200 mg) or 200ml (400 mg).

12.12.2 Preparation: For parenteral administration, the commercial solution for injection is available in its final form for administration (concentration of 2 mg/ml).

12.12.3 Storage and Stability: Oral tablets and oral suspension should be stored at temperatures below 30ºC (86ºF). Store reconstituted oral suspension between 5º to 30ºC (41º to 86ºF) and discard unused portion after 2 weeks. Fluconazole for injection in glass bottles should be stored between 5º to 30ºC (41º to 86ºF). Fluconazole for injection in Viaflex® Plus plastic containers should be stored between 5º to 25ºC (41º to 77ºF).

12.12.4 Administration: Oral and parenteral. Parenteral doses should be administered by an intravenous infusion at a maximum rate of 200mg/hr. e) Toxicities: 1) Nausea, vomiting, headache, skin rash, abdominal pain, diarrhea have been reported at an incidence of 1% or greater in clinical trials. 2) In combined clinical trials and marketing experience, there have been rare cases of serious hepatic reactions during treatment with fluconazole. The spectrum of these hepatic reactions has ranged from mild transient elevation in transaminases to clinical hepatitis, cholestasis and fulminant hepatic failure, including fatalities.

12.12.5 Drug Interactions: Fluconazole is a potent inhibitor of the cytochrome P450 3A4 isoenzyme system. Coadministration of fluconazole with other drugs metabolized by the same enzyme system may result in increased plasma concentrations of the drugs, which could increase or prolong therapeutic and adverse effects. Refer to the package literature or other drug information resources for additional information on identification and management of potential drug interactions.

12.13 TRIMETHOPRIM/SULFAMETHOXAZOLE (TMP/SMX, COTRIMOXAZOLE, BACTRIM, SEPTRA)

12.13.1 Supply: Commercially available as a single strength tablet containing trimethoprim 80mg and sulfamethoxazole 400mg and a double strength (DS) tablet containing trimethoprim 160mg and sulfamethoxaole 800mg. It is also available in a oral suspension at a concentration of 40mg of trimethoprim and 200mg sulfamethoxazole per 5ml. Parenteral TMP/SMX is available in a solution for injection at a concentration of 80mg of trimethoprim and 400mg of sulfamethoxazole per 5ml.

12.13.2 Preparation: For parenteral administration, the commercial solution for injection must be diluted prior to administration. It is recommended that each 5ml of the solution for injection be diluted with 100-125 ml or, if fluid restriction is required, in 75ml of dextrose 5% in water. 0.9% NaCl may be substituted as a diluent but the resulting

solutions have reduced stability. Consult with pharmacy for questions regarding diluent, volume, and expiration.

12.13.3 Storage and Stability: Oral tablets and oral suspension should be stored at 15º to 30ºC (59º to 86ºF) in a dry place and protected from light. TMP/SMX for injection should be stored at room temperature between 15º to 30ºC (59º to 86ºF) and should not be refrigerated. Stability of intravenous doses after final dilution is dependent on concentration and diluent. Consult with pharmacy for questions regarding stability and expiration dating.

12.13.4 Administration – Oral and parenteral. Parenteral doses should be administered by an intravenous infusion over 60 to 90 minutes.

12.13.5 Toxicities: 1) The most common adverse effects from TMP/SMX are gastrointestinal disturbances (nausea, vomiting, anorexia) and allergic skin reactions (such as rash and urticaria). 2) Fatalities associated with the administration of sulfonamides, although rare, have occurred due to severe reactions, including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia and other blood dyscrasias. 3) For TMP/SMX injection, local reaction, pain and slight irritation upon IV administration are infrequent. Thrombophlebitis has rarely been observed.

12.14 MEDROXYPROGESTERONE ACETATE (PROVERA)

12.14.1 Supply: Commercially available as 2.5mg, 5mg, and 20mg tablets.

12.14.2 Storage: Tablets should be stored at controlled room temperature 20º to 25ºC (68º to 77ºF).

12.14.3 Administration: Oral.

12.14.4 Toxicities include nausea, breast tenderness or galactorrhea, sensitivity reactions (urticaria, pruritis, edema, rash), acne, alopecia, hirsutism, fluid retention, mental depression, cholestatic jaundice, changes in cervical erosion and cervical secretions, breakthrough bleeding or spotting, and thromboembolic phenomena.

12.15 IMMUNE GLOBULIN INTRAVENOUS (IGIV)

12.15.1 Supply: Commercially available (multiple sources) in a variety of formulations, including solution for injection and powder for injection in a variety of vial sizes.

12.15.2 Preparation: Refer to the manufacturer's package labeling for guidelines in preparation.

12.15.3 Storage and Stability: Refer to the manufacturer's package labeling for storage and stability information.

12.15.4 Administration: Intravenous infusion. Refer to the manufacturer's package labeling for recommendations on rate of administration.

12.15.5 Toxicities: 1) Infusion related toxicities may occur. Symptoms may include flushing, feelings of tightness in the chest, chills, fever, dizziness, nausea, diaphoresis, backache, leg cramps, urticaria, and hypotension or hypertension. Slowing or stopping the infusion usually allows the symptoms to disappear promptly. 2) Immediate anaphylactic and hypersensitivity reactions are a remote possibility. Patients with IgA deficiency who

have developed antibodies to IgA have an increased risk of anaphylactic reactions when treated with IgA-containing IGIV products. 3) Immune Globulin Intravenous products have been associated with renal dysfunction, acute renal failure, osmotic nephrosis, and death. Patients predisposed to acute renal failure include patients with any degree of pre-existing renal insufficiency, diabetes mellitus, > 65 years of age, volume depletion, sepsis, paraproteinemia, or patients receiving known nephrotoxic drugs. Especially in such patients, IGIV products should be administered at the minimum concentration available and the minimum rate of infusion practicable. While these reports of renal dysfunction and acute renal failure have been associated with the use of many of the licensed IGIV products, those containing sucrose as a stabilizer accounted for a disproportionate share of the total number. 4) Since Immune Globulin Intravenous products are made from human plasma, there is the risk of transmitting infectious agents such as viruses that can cause disease. This risk has been reduced by screening plasma donors, testing for certain virus infections, and by inactivating and/or removing certain viruses. 5) An aseptic meningitis syndrome has been reported to occur infrequently in association with IGIV treatment. 6) There is also clinical evidence of a possible association between IGIV administration and thrombotic events. The exact cause of this is unknown; therefore caution should be exercised in the prescribing and infusion of IGIV in patients with a history of cardiovascular disease or thrombotic episodes.

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14 APPENDICES

14.1 APPENDIX A: PROPHYLAXIS AND TREATMENT OF INFECTIOUS COMPLICATIONS

* Note: It is recommended that investigators also refer to the *Guidelines for Infection Management in Allogeneic Hematopoietic Stem Cell Transplant Recipients*, as this source may have information that reflects more current recommendations. This source can be found at: http://intranettst2.cc.nih.gov/bmt/clinicalcare/infectionmanagement.shtml

a) Pneumocystis carinii pneumonia

- At the start of pre-transplant induction chemotherapy, all patients will receive trimethoprim 160 mg/sulfamethoxazole 800 mg (TMP/SMX 160/800), one tablet PO BID on two days per week (e.g., on each Saturday and Sunday). This will continue until completion of induction chemotherapy.
- Prophylaxis is resumed post-transplant when the absolute neutrophil count is greater than 1000/µl and the platelet count is consistently above 50,000/mm^3 for two consecutive days without transfusion.
- The dose for post-transplant prophylaxis is TMP/SMX 160/800, one tablet PO BID on two days per week, continuing for 6 months after transplantation or until the patient is off immunosuppression, whichever is longer.
- For patients with sulfa allergy, pentamidine inhalation, 300 mg every four weeks may be utilized.

b) Yeast Infections

- On day 6 of the first cycle of pre-transplant induction chemotherapy, all patients will begin fluconazole 400 mg PO daily. Because of its interaction with vincristine, fluconazole will be discontinued during days 1, 2, 3, 4 and 5 of induction chemotherapy.
- Fluconazole will be restarted on day 6 of each cycle.
- Patients will continue fluconazole 400 mg PO daily throughout the transplant conditioning regimen (day –6) until 100 days after transplantation or until the patient is off immunosuppression, whichever is longer.
- Intravenous dosing will be substituted when patients are unable to tolerate oral medications.

c) Mold Infections (including *Aspergillus*)

Neutropenic patients who are febrile for more than 5 days despite broad-spectrum antibiotics and have no clinical evidence of invasive fungal disease should be considered candidates to receive either liposomal amphotericin B (Ambisome; 5 mg/kg/day) or voriconazole (6 mg/kg q12h for two doses and then 4 mg/kg q12h). If initiated, such therapy would typically continue until the patient attains an ANC > 500/µl AND is afebrile for 24 hours. Fluconazole prophylaxis will be discontinued during this therapy.

d) Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV)

- All patients will receive valacyclovir (500 mg PO QD) for suppression of HSV and VZV infection/reactivation.
- This prophylaxis will begin when patients start induction therapy (EPOCH-F/R) and continue throughout transplantation. Valacyclovir will continue for 6 months after transplantation or until the end of immunosuppression, whichever is longer.

- If a patient cannot take oral medications, acyclovir 250 mg/m^2 IV q12h will be substituted for valacyclovir.

e) Cytomegalovirus (CMV)

- CMV monitoring and therapy will be according to the continuing updated guideline provided by the NCI Infectious Disease website maintained by Dr. Juan Gea-Banacloche at: http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml

f) Bacterial Pathogens

- All patients receiving treatment for chronic GVHD will receive penicillin VK (250 mg PO BID) until treatment for chronic GVHD is discontinued. For penicillin-allergic patients, TMP/SMX 80/400 PO QD can be used. For patients allergic to both penicillin and sulfa, clarithromycin 500 mg PO QD can be used.
- All patients will receive *S. pneumoniae* 23-valent polysaccharide vaccine (Pneumovax) at 12 and 24 months, and Hib vaccine at 12, 14 and 24 months after transplant.

14.2 APPENDIX B: CLASSIFICATION AND TREATMENT OF ENGRAFTMENT SYNDROME

- Neutrophil recovery after transplantation may result in "engraftment syndrome" (which may include fever, rash, edema, hypoxia)
- The following criteria will be utilized to diagnose engraftment syndrome (must have 3 major criteria or 2 major + 1 minor criteria):

Major criteria:	- Fever $\geq$ 38.3° with no identifiable infectious etiology - Erythrodermatous rash (25% of BSA; not due to medication) - Noncardiogenic pulmonary edema (pulmonary infiltrates/hypoxia)
Minor criteria	- Hepatic dysfunction (total bilirubin $\geq$ 2 mg/dl or transaminases levels $\geq$ two times normal) - Renal insufficiency (serum creatinine > two times baseline) - Weight gain > 2.5% of baseline body weight - Transient encephalopathy unexplainable by other causes

- To promote consistency of our clinical practice, the following will be the recommended therapy for engraftment syndrome. However, this schedule may be modified (taper either accelerated or delayed) for individual patients as clinical circumstances warrant.

Day 1: Methylprednisolone (MP) 250 mg IV Q6 hr x 4 doses
Day 2: MP 250 mg IV Q8 hr x 3 doses
Day 3: MP 250 mg IV Q12 hr x 2 doses
Day 4: MP 125 mg IV Q12 hr x 2 doses
Day 5: MP 60 mg IV Q12 hr x 2 doses
Day 6: MP 30 mg IV Q12 hours x 2 doses

Days 7-8: Prednisone 60 mg PO QD x 2 days
Days 9-10: Prednisone 50 mg PO QD x 2 days
Days 11-12: Prednisone 40 mg PO QD x 2 days
Days 13-14: Prednisone 30 mg PO QD x 2 days
Days 15-16: Prednisone 20 mg PO QD x 2 days
Days 17-18: Prednisone 10 mg PO QD x 2 days
Day 19: Discontinue prednisone

14.3 APPENDIX C: GRADING AND MANAGEMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE

Clinical Staging

Stage	Skin	Liver	Gut
+	Rash < 25% BSA	Total bilirubin 2-3 mg/dl	Diarrhea 500-1000ml/d
++	Rash 25-50% BSA	Total bilirubin 3-6 mg/dl	Diarrhea 1000-1500ml/d
+++	Generalized erythoderma	Total bilirubin 6-15 mg/dl	Diarrhea >1500ml/d
++++	Desquamation and bullae	Total bilirubin > 15 mg/dl	Pain +/- ileus

BSA = body surface area; use "rule of nines" or burn chart to determine extent of rash.

Clinical Grading

	Stage			
Grade	**Skin**	**Liver**	**Gut**	**PS**
0 (none)	0	0	0	0
I	+ to ++	0	0	0
II	+ to +++	+	+	+
III	++ to +++	++ to +++	++ to +++	++
IV	++ to ++++	++ to ++++	++ to ++++	+++

Treatment

This schema is intended to serve as a guideline and to promote consistency in our clinical practice; it may be modified for individual patients as clinical circumstances warrant.

Grade 0-I GVHD

1) Topical corticosteroids (usually 0.1% triamcinolone; 1% hydrocortisone to face) applied to rash BID.

Grade II-IV GVHD

1) Methylprednisolone (MP) 62.5 mg/m^2 per dose IV, BID for 4 consecutive days.
2) If no response after 4 days, continue until response (7-day maximum trial).
3) If response within 7 days, taper as follows:
   - 50 mg/m^2 per dose IV BID for 2 days.
   - 37.5 mg/m^2 per dose IV BID for 2 days.
   - 25 mg/m^2 per dose IV BID for 2 days.
   - If clinically appropriate, change MP to oral prednisone 100 mg PO (or oral equivalent of IV dose) daily for 2 days. MP may be converted to prednisone later in the taper at the investigators' discretion.
   - After this, steroids will be reduced by 10% each week until a dose of 10 mg/day is reached. Subsequent reductions will be made at the investigators' discretion.
   - If GVHD worsens during taper, steroids should be increased to previous dose.

   During steroid taper, maintain therapeutic cyclosporine levels (200 to 250 ng/ml).
4) If no response is observed within 7 days of MP treatment

- Increase Methylprednisolone to 500 mg/m^2 per dose IV, BID for 2 days.
- If there is no improvement, consideration will be given to using second-line immunosuppressive therapy, e.g., tacrolimus, mycophenolic acid, monoclonal antibodies, or studies of investigational agents for acute GVHD, if they are available.

5) Antifungal prophylaxis with agents effective against mould will be started when it is anticipated that the patient will be receiving steroids at $\geq$ 1 mg/kg/d of methylprednisolone (or equivalent) for $\geq$ 2 weeks. Voriconazole is the agent of choice, but liposomal amphotericin B (Ambisome) 5 mg/kg/d or amphotericin B lipid complex (Abelcet) 5 mg/kg/d are valid alternatives. During prophylaxis with any of the above agents, fluconazole should be discontinued. In patients with therapeutic cyclosporine levels at the initiation of voriconazole therapy, the cyclosporine dose should be decreased by approximately 50%.

6) Determination of GVHD treatment response should be made within 96 hours of starting therapy. The following are criteria to determine response to GVHD treatment:

- Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD.
- Partial Response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of adequate performance status (Karnofsky Score $\geq$ 70%).
- Non-responder: < 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Failure to maintain adequate performance status (Karnofsky Score $\leq$ 70%).
- Progressive disease: Further progression of signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

14.4 APPENDIX D: DATA COLLECTION ELEMENTS REQUIRED BY PROTOCOL

All of the following elements will be recorded in the C3D database.

A. PATIENT ENROLLMENT

- Date of birth, age, gender, race, ethnicity
- Height
- Weight
- Performance Status
- Date of original diagnosis (in years)
- Stage at diagnosis
- Stage at study entry
- Sites of disease at diagnosis and study entry
- Tumor Histology and date of confirmation
- Date of Informed Consent signature, consent version and date of registration
- Baseline History/Physical (day 14 post-transplant is considered baseline)
- Baseline Symptoms (day 14 post-transplant is considered baseline; only grade 2 symptoms and above need to be recorded as baseline symptoms)
- Prior therapy (name of regimen only; doses not needed)
- Prior surgery (not including minor surgeries such as bone marrow biopsy)
- Findings of consultations done at screening

B. LABORATORY AND DIAGNOSTIC TEST DATA

- All Clinical laboratory and diagnostic test results done at screening and until day 100 post transplant with the following exceptions:
- Diagnostic tests which are not specified in the protocol, and if the results are not needed to document the start or end of an adverse event that requires reporting.
- All clinical laboratory and diagnostic tests done after day 100 that support a possible, probable or definite diagnosis of GVHD, infection or secondary malignancy and those done to document a change in grade and the end of these adverse events.
- All tests done to document resolution of adverse events that occurred in the first 100 days post-transplant
- HLA data (patient and donor).
- Serologies-CMV, HSV, EBV, toxoplasmosis, adenovirus (patient and donor)
- TTV data (patient and donor)
- Blood, bone marrow, and tumor chimerism data. For blood chimerism, it is only necessary to capture day 14, day 28, day 100 and day 180 values for CD3+ T cell chimerism and CD15+ myeloid cell chimerism.

C. ADVERSE EVENTS

- Adverse events will be collected after initiation of experimental therapy (day 14 post-transplant).

- As such, day 14 post-transplant will be considered the baseline for AE recording.
- Grade 3 and 4 adverse events will be recorded with the following exceptions:
    - Grade 3 and 4 lymphopenia, leukopenia, bone marrow cellularity, CD4 count,
    - Grade 3 platelets and neutropenia
- The following grade 2 adverse events will be recorded:
  Toxicities potentially associated with GVHD
  Toxicities potentially attributable to the Th2 cells
  Toxicities potentially related to infection.
- GVHD data to include: maximal grade, time of onset, sites of involvement, and response to therapy.
- After day 100 post transplant adverse events will only be recorded if potentially associated with the Th2 cells, opportunistic infection, GVHD, or secondary malignancy. Adverse events including those associated with non-study drugs, chemotherapy, radiation, and surgery after day 100 will not be recorded unless they constitute serious adverse events requiring expedited adverse event reporting as defined by the IRB.

- Adverse event recording will focus on transplant-related parameters, in particular GVHD and infection. Toxicities attributable to GVHD, infection, or the Th2 cell therapy will be recorded.
- Because chemotherapy sensitivity is an important aspect of the randomization, it will also be important to capture disease response at the following time points: just prior to administration of the preparative regimen, at days 28, 100, and 180 post-transplant, and then at the yearly time points.

- For the protocol consideration and C3D database consideration, standard of care treatment that involves further high-dose chemotherapy and donor stem cell infusion will be considered a post-transplant standard of care therapeutic intervention. As such, the specific medications used for the intervention (such as type of immune suppression) and the specific time points of patient monitoring (such as chimerism; such as radiology studies) will be determined by the PI/LAI and detailed and discussed at the weekly protocol meetings.
- However, interventions that meet the standard CIBMTR definition of a "second transplant" (for example, high-dose chemotherapy plus stem cell support to treat graft rejection) will need to have protocol notes that allow completion of the CIBMTR data reporting forms for a new transplant procedure (in particular, re-starting the "clock" in terms of days post-transplant nomenclature).
- It will not be necessary to maintain a radiation history CRF.
- Concomitant medications should be captured in terms of post-transplant use of immune suppressive drugs (in particular, prednisone).

D. CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY

- Completion of protocol therapy
- Patient requests to be withdrawn from protocol therapy
- Initiation of additional or alternative standard of care treatments
- Investigator discretion

- Positive pregnancy test

E. OFF STUDY

- Date and reason for off study
- Date and cause of death
- Autopsy findings
- PI decision to end this study
- Donor subjects who become decisionally impaired

Note: Typically, at 5-years post-transplant, patients can be transferred to an ETIB natural history study rather than stay on this treatment protocol. However, for patients with substantial chronic GVHD or malignancy concerns, it may be preferable to remain on this treatment protocol.

14.5 APPENDIX E: CHRONIC GVHD

Chronic GVHD will be scored according to the NIH Consensus Criteria [Filipovich, AH et al; NIH consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biology of Blood and Marrow Transplantation, 2005. 11(12): pages 945-956.]

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN
LUNGS* **FEV1** ☐ **DLCO** ☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring 0_2) ☐ FEV1 ≤39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

keratoconjunctivitis sicca

***** AP may be elevated in growing children, and not reflective of liver dysfunction

Other indicators, clinical manifestations or complications related to cGVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact (none – 0,mild -1, moderate -2, severe – 3)

ĺ Esophageal stricture or web___ ĺ Pericardial Effusion___ ĺ Pleural Effusion(s)___
ĺ Ascites (serositis)___ ĺ Nephrotic syndrome___ ĺ Peripheral Neuropathy___
ĺMyasthenia Gravis___ ĺ Cardiomyopathy___ ĺ Eosinophilia > 500µl___
ĺ Polymyositis___ ĺ Cardiac conduction defects___ ĺ Coronary artery involvement___
ĺ Platelets <100,000/µl___ ĺ Progressive onset_____ Others_____