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PROTOCOL TITLE: A Pilot Study of EPOCH-F/R Induction Chemotherapy and Reduced-Intensity, HLA-Matched, Related Allogeneic Hematopoietic Stem Cell Transplantation for Refractory or Relapsed Hematologic Malignancies, with Cyclosporine & Methotrexate for Graft-Versus-Host Disease Prophylaxis

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Precis

Allogeneic hematopoietic stem cell transplantation (HSCT) is potentially curative for refractory hematologic malignancies, but its application has been limited historically by morbidity and mortality from conventional transplant preparative regimens and graft-versus-host disease (GVHD). Donor T cells mediate GVHD and also help to eradicate malignancies through an immune-dependent graft-versus-tumor effect. Efforts to decrease preparative regimen toxicity have led to reduced-intensity, or "nonmyeloablative" regimens, facilitating the study of allogeneic HSCT in a broader population. As a promising strategy for reducing GVHD, donor Th2 cells were shown to abrogate Th1-mediated GVHD without impairing engraftment in murine models of allogeneic HSCT. These findings led to a phase I/II clinical study of donor Th2 cells for the prevention of GVHD during reduced-intensity allogeneic HSCT (CC 99-C-0143); preliminary results suggest that a randomized trial will be necessary to evaluate donor Th2 cells further.

In CC 99-C-0143, a novel induction chemotherapy regimen, EPOCH-Fludarabine (EPOCH-F), was well tolerated and effective for sequential host immune depletion. However, a significant proportion of patients failed to achieve satisfactory disease control before transplant, providing a basis for intensifying this induction regimen. Furthermore, the initial 20 patients treated on this study experienced relatively high rates of acute GVHD and considerable morbidity associated with cyclosporine monotherapy for GVHD prevention, indicating that future studies should use more aggressive prophylaxis. These observations warrant modifying our approach to allogeneic HSCT before undertaking a randomized study of donor Th2 cells.

We now propose a pilot study of HLA-matched, related, reduced-intensity allogeneic HSCT in refractory hematologic malignancies, using an intensified EPOCH-F induction chemotherapy regimen with rituximab added for patients with CD20+ malignancies (EPOCH-F/R). This regimen will be evaluated for toxicity and disease control before transplantation. GVHD prophylaxis will consist of a standard dual-agent regimen, cyclosporine/methotrexate; the impact of this change on hematopoietic recovery, donor/recipient chimerism, and the incidence of acute GVHD will be assessed.

Immune reconstitution following allogeneic HSCT is an important research interest among Experimental Transplantation and Immunology Branch investigators. Current evidence suggests a critical role for interleukin-7 (IL-7) in CD4+ T cell homeostasis, and interleukin-15 (IL-15) appears crucial to CD8+ T cell and NK cell homeostasis. The relationships between these cytokines and lymphocyte subpopulations have not been studied in the setting of allogeneic HSCT; such analysis may enhance our understanding of engraftment kinetics, graft-versus-host disease, and immune reconstitution. We will correlate serum IL-7 and IL-15 levels with changes in circulating T-cell and NK-cell subpopulations during EPOCH-F/R induction chemotherapy, after transplantation, and with the development of GVHD.

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1.0 Introduction

1.1 Study Objectives:

1.1.1 Primary Objectives:

a) To evaluate the effectiveness of cyclosporine and mini-dose methotrexate (CSA/mini-MTX) as prophylaxis for acute graft-versus-host disease (GVHD) following allogeneic HSCT, after induction chemotherapy with EPOCH-F/R and reduced-intensity conditioning with cyclophosphamide and fludarabine.

1.1.2 Secondary Objectives:

a) To study the toxicity of a modified induction chemotherapy regimen, EPOCH-Fludarabine/Rituximab (EPOCH-F/R), prior to allogeneic hematopoietic stem cell transplantation (HSCT). This evaluation will be made informally in comparison with the toxicity observed for the EPOCH-F regimen employed in CC 99-C-0143.

b) To assess the impact on hematologic recovery and donor/recipient chimerism of cyclosporine and mini-dose methotrexate for GVHD prophylaxis in the setting of reduced-intensity allogeneic HSCT following sequential immunoablative chemotherapy.

c) To evaluate the effectiveness of EPOCH-F/R with respect to anti-tumor activity and sequential host immune depletion prior to allogeneic HSCT.

d) To correlate serum interleukin-7 and interleukin-15 levels with *in vivo* changes in host lymphocyte subpopulations, both during sequential immunoablative chemotherapy before allogeneic HSCT and during immune reconstitution after transplantation.

e) To determine the overall response rate to salvage therapies administered for disease progression after allogeneic HSCT.

1.2 Background and Rationale:

1.2.1 *Reduced-Intensity Preparative Regimens in Allogeneic HSCT:*

Conventional transplantation regimens typically contain high doses of alkylating agents often in combination with total body irradiation. The use of such regimens, which are considered myeloablative, was once perceived as essential both for eradication of malignant disease and for suppression of the host-versus-graft marrow rejection response; however, recent studies have challenged both of these assumptions. The high frequency of relapse observed in the setting of T-cell-depleted allogeneic

HSCT suggests that the curative potential of allogeneic HSCT results primarily from a T-cell-mediated graft-versus-tumor (GVT) effect, and not from the myeloablative doses of chemotherapy [1]. In addition, the delayed administration of donor lymphocytes can induce complete remissions remote from the preparative regimen; this clinical observation provides further evidence that an immune mechanism primarily mediates the anti-leukemic effects of allogeneic HSCT [2]. With respect to graft rejection, it is also now known that conventional myeloablative regimens do not consistently eliminate the host response to the allograft, even in recipients of HLA-matched allografts. For example, 30 to 40% of patients will reject a T-cell-depleted, HLA-matched marrow graft after myeloablative conditioning [3]. These limitations of myeloablative preparative regimens, combined with the high levels of morbidity and mortality associated with myeloablation, result in a low therapeutic index for this aspect of allogeneic HSCT. It has therefore been a goal of clinical investigators to develop regimens that reduce treatment mortality yet maintain the efficacy of allogeneic HSCT, by establishing allo-engraftment without myeloablation.

Recent allogeneic HSCT studies have demonstrated that fludarabine can contribute to the prevention of marrow rejection in reduced-intensity, or "nonmyeloablative", transplant approaches [4, 5]. In the non-transplant setting, fludarabine can cause immunosuppression through its depletion of both $CD4^+$ and $CD8^+$ T cells [6]. Importantly, patients receiving fludarabine-containing combination chemotherapy are susceptible to developing transfusion-associated graft-versus-host disease [7]. This observation illustrates the potential of fludarabine-based regimens for preventing the rejection of even HLA-disparate lymphoid cells; such regimens may be considered "immunoablative", in recognition of their relatively specific impact upon host cell-mediated immunity.

Unfortunately, published reports indicate that allogeneic HSCT using such reduced-intensity regimens has been limited by incomplete donor chimerism or failure to engraft, which were associated with relapsed malignancy and higher rates of death due to progressive disease [4, 5, 8]. For these reasons, we believe that attaining full donor chimerism early after transplantation is central to achieving an enhanced GVT effect, and that accomplishing this goal with a reduced-intensity preparative regimen depends upon the degree of host immunosuppression at the time of HSCT.

1.2.2 *Donor Th2 Cells As a Strategy to Reduce Graft-Versus-Host Disease Following Allogeneic HSCT:* Donor T cells in the allograft are the primary initiators of GVHD; this complication can be prevented by depletion of T cells from the allograft, but such T-cell-depleted transplants are associated with a high rate of graft rejection. As an alternative approach, we have evaluated whether donor T cells of defined cytokine

phenotypes might differentially mediate allogeneic transplantation responses. A type I immune response, mediated by $CD4^+$, Th1 and $CD8^+$, Tc1 cells, is characterized by the secretion of the pro-inflammatory cytokines IL-2 and IFN-gamma [9]. In contrast, the secretion of the anti-inflammatory cytokines IL-4, IL-5, and IL-10 characterizes a type II immune response, mediated by CD4+, Th2 and CD8+, Tc2 cells. Given that acute GVHD is typical of a type I immune response (characterized by an initial phase of IL-2 production, and followed by IFN-γ secretion and cytolytic function) [10], we hypothesized that donor T cells of type I cytokine phenotype would generate acute GVHD, and that donor T cells of type II cytokine phenotype would regulate GVHD. In murine models, we demonstrated that donor $CD4^+$ T cells of Th1 cytokine phenotype (defined by their secretion of IL-2 and IFN-γ) mediated GVHD. In contrast, donor $CD4^+$ T cells of Th2 phenotype (defined by their secretion of IL-4, IL-5, and IL-10) did not generate GVHD, and abrogated the ability of other donor T cell populations to initiate GVHD [11]. Importantly, we also demonstrated in murine models that Th2-mediated regulation of GVHD was not associated with an increased rate of graft rejection [12]. Thus, the supplementation of marrow allografts with donor Th2 cells represents a strategy for reducing the detrimental aspect of allogeneic T cell administration (GVHD) while preserving the beneficial ability of donor T cells to prevent allograft rejection. The clinical evaluation of this strategy is ongoing in a phase II NCI trial (CC 99-C-0143); we anticipate that a randomized study will follow for further assessment of the impact of donor Th2 cell administration on GVHD, engraftment, and anti-tumor effects. An important aspect of the design for the current protocol proposal is the dual-agent GVHD prophylaxis regimen we will use (Section 1.2.8), which will serve as a control group for future randomized studies involving donor Th2 cells.

1.2.3 *Rationale for EPOCH-Fludarabine (EPOCH-F) Induction Prior to Reduced-Intensity Conditioning in CC 99-C-0143:* The desire to achieve rapid and complete donor chimerism following allogeneic HSCT led our group to adopt a strategy of sequential immune depletion before transplant conditioning. In CC 99-C-0143, we developed a novel induction chemotherapy regimen consisting of fludarabine in combination with the agents contained in the EPOCH regimen. The main purpose of administering this induction regimen was to achieve a high level of host immunosuppression prior to allogeneic HSCT, in order to attain rapid conversion to full donor chimerism and to facilitate the development of a GVT effect soon after transplantation. Murine data indicate that very severe levels of host T cell depletion are required for the engraftment of fully-MHC mismatched allografts after fludarabine-based chemotherapy [13]. Thus, we believe the development of induction chemotherapy

regimens that induce severe host T cell depletion without myeloablation is a desirable goal. As a surrogate marker for the level of immune depletion necessary to achieve this goal in CC 99-C-0143, we chose a peripheral blood CD4 count of less than 50 cells/μl before administration of the transplant preparative regimen. Our hypothesis was that this level of host $CD4^+$ T cell depletion, which is associated with clinically significant immunosuppression and a reduced ability to reject allogeneic cells in patients with B cell malignancy [7, 14, 15], would predict for robust engraftment after allogeneic HSCT and be attainable with up to 3 cycles of induction chemotherapy before transplantation.

Developed in the NCI Medical Oncology Clinical Research Unit, EPOCH is a well-tolerated chemotherapy regimen with established activity in refractory lymphoid malignancies [16-18]. Fludarabine, which has modestly immunosuppressive effects when administered as a single agent, can be highly immunosuppressive when given in combination with steroids, alkylators, or topoisomerase II inhibitors [19-21]; all of these categories of antineoplastic agents are represented in the EPOCH regimen. These observations suggested that the combination of fludarabine and EPOCH chemotherapy might result in severe host T cell depletion at the time of transplantation and have clinical activity in refractory hematologic malignancies.

1.2.4 *EPOCH-F Results in CC 99-C-0143 – Disease Control*. Through July 2002, 48 patients with refractory and/or relapsed hematologic malignancies received EPOCH-F on CC 99-C-0143.

Diagnosis	**(n)**
Diffuse large B cell lymphoma	15
Follicular NHL	6
Chronic lymphocytic leukemia (1 prolymphocytic, 1 Richter's)	6
Multiple myeloma	6
Mantle cell lymphoma	4
Hodgkin's lymphoma	3
Chronic myelogenous leukemia	3
Myelodysplasia	3
Acute lymphocytic leukemia	1
Lymphomatoid granulomatosis	1

Patients had received a median of 3 prior therapies before enrolling (range, 1-6). 20 patients had primary refractory disease; 22 were in relapse, of whom 18 were refractory. Only 2 were in remission at study entry.

Responses to EPOCH-F induction are shown in the following table:

	(n)	%
Complete response	3	6
Partial response	15	31
Stable disease	15	31
Mixed response	1	2
Progressive disease	14	29

Thus, EPOCH-F was associated with an overall response rate of 37% in this relatively chemoresistant patient population, while nearly one-third of patients developed progressive disease while receiving this induction regimen. Analysis of the 15 patients with diffuse large B cell lymphoma indicates that at study entry, 6 had primary refractory disease, 7 were in refractory relapses, and 2 were in complete remission. After receiving EPOCH-F, 8 patients had disease progression, 5 had stable disease, and 1 achieved a partial response. 1 patient remained in CR that existed before study entry. Therefore, results in this subgroup were consistent with those of the overall study population.

1.2.5 *EPOCH-F Results in CC 99-C-0143 – Immune Depletion*. 25 patients received 3 cycles of EPOCH-F before transplantation, the maximum number permitted by the protocol. 11 patients received only 2 cycles, and 12 patients received only 1 cycle. Reasons for proceeding to transplantation after fewer than 3 cycles of EPOCH-F included: having reached CD4 < 50/μl (n=8); progressive disease on EPOCH-F (n=11); withdrawal from study (n=1, not transplanted); unacceptable toxicity from EPOCH-F (n=3). The effect of EPOCH-F induction on circulating lymphocyte subpopulations is shown in the following table:

	Median CD4 (range)	Median CD8 (range)	Median ALC (range)
Baseline	283 (9-1697)	261 (13-3478)	837 (37-282,854)
Post cycle 1	212 (12-1004)	117 (5-1484)	582 (68-196,770)
Post cycle 2	151 (39-388)	75 (17-571)	487 (1-177,742)
Post cycle 3	97 (48-309)	52 (2-830)	393 (141-171,952)
Pre-transplant	89 (12-452)	52 (2-830)	380 (1-171,952)

Although the goal CD4 count of less than 50 cells/μl was reached in only 14 of 48 patients following induction, all patients who underwent transplantation demonstrated evidence of predominantly donor engraftment, with only one patient having < 95% donor chimerism at day +28 following transplantation. Thus, the inability to reach a CD4 target of 50 cells/μl in all patients was not an impediment to engraftment in CC 99-C-0143; in fact, 24 of these patients had CD4 counts > 100/μl before receiving the transplant conditioning regimen. This outcome suggests that as a surrogate marker for immune depletion, the CD4 target of 50/μl was lower than necessary to ensure early engraftment with full donor

chimerism in the setting of a T-cell-replete, reduced-intensity allogeneic HSCT. It therefore follows that some patients with stable or responsive disease received more cycles of immune-depleting induction chemotherapy than was required to ensure engraftment, resulting in delays before transplantation for these patients. Choosing a higher CD4 target might permit some patients to proceed to transplant with fewer cycles of induction therapy; this would be of particular benefit to patients with minimal disease or stable disease during EPOCH-F.

1.2.6 *EPOCH-F Results in CC 99-C-0143 – Toxicity*. EPOCH-F was a well-tolerated regimen in this population, with no treatment-related deaths attributed to the induction therapy. In 109 cycles of EPOCH-F, only 7 patients experienced grade 4 non-hematologic toxicity, including anorexia (n=4), vomiting (n=1), catheter-related sepsis (n=1), and viral encephalitis (n=1). Grade 4 hematologic toxicity occurred in 16 patients and lasted a median of 4 days per episode (range, 2-8 days). These included 24 episodes of neutropenia, of which 4 were associated with documented infections. There were also 2 episodes of grade 4 thrombocytopenia.

Grade 3 non-hematologic toxicity affected 28 patients and lasted a median of 4 days, including 12 episodes of neutropenic fever and 6 documented infections (of which 2 coincided with grade 3 or 4 neutropenia). Other grade 3 events were sporadic, including: hypoxia, motor neuropathy, neuropathic pain, and hyperbilirubinemia (2 episodes each); sensory neuropathy, hyponatremia, syncope, anorexia, diarrhea, hypotension, urinary frequency, thrombotic microangiopathy, bone pain, rectal pain, pain-other (1 episode each).

To summarize, results from CC 99-C-0143 indicate that the EPOCH-F induction regimen provides disease control or stability in a majority of patients with refractory or relapsed hematologic malignancies, with an acceptable toxicity profile. However, the fact that nearly one-third of patients had disease progression while receiving EPOCH-F provides a strong rationale for attempting to intensify this induction regimen, given the importance of disease control before transplantation in the reduced-intensity setting. The generally mild and reversible nature of toxicities observed with EPOCH-F suggests that intensification of this induction regimen could be achieved without an unacceptable increase in adverse events.

1.2.7 *EPOCH-Fludarabine/Rituximab (EPOCH-F/R) Induction for Disease Control and Sequential Immune Depletion before Reduced-Intensity Allogeneic HSCT:* Consistent with the above, in the current study we will pilot a new induction chemotherapy regimen based upon the EPOCH-F regimen used in CC 99-C-0143. In that protocol, the EPOCH component of the induction regimen was an attenuated version of EPOCH as used in

previous NCI Medical Oncology Clinical Research Unit studies [16-18]. Specifically, our EPOCH regimen (as a part of EPOCH-F) differed from other EPOCH protocols in that the infusional component was reduced from four days to three, prednisone was reduced from five days to four, and cyclophosphamide was given on day 4 (instead of day 5) at a 20% lower dose. These changes were made to allow for the possibility of added toxicity from the addition of fludarabine to the EPOCH regimen.

The differences between the EPOCH-F induction regimen of CC 99-C-0143 and the current proposal are outlined as follows, with changes underlined:

CC 99-C-0143 EPOCH-F	**Current Proposal EPOCH-F/R**
	Rituximab 375 mg/m^2 IV, day 1 (for patients with CD20+ malignancies)
Fludarabine 25mg/m^2 IV QD, days 1-3	Fludarabine 25mg/m^2 IV QD, days 1-4
Etoposide 50 mg/m^2 Doxorubicin 10 mg/m^2 Vincristine 0.5 mg/m^2 } CIV QD, days 1-3	Etoposide 50 mg/m^2 Doxorubicin 10 mg/m^2 Vincristine 0.4 mg/m^2 } CIV QD, days 1-4
Prednisone 60 mg/m^2 PO QD, days 1-4	Prednisone 60 mg/m^2 PO QD, days 1-5
Cyclophosphamide 600 mg/m^2 IV, day 4	Cyclophosphamide 750 mg/m^2 IV, day 5

The increases in prednisone, cyclophosphamide, and the infusional component of EPOCH (etoposide, doxorubicin, and vincristine) are intended to achieve the full dose intensity of other NCI MOCRU protocols employing EPOCH chemotherapy. Increasing fludarabine from three to four days of administration is expected to potentiate both disease control (many of the patients in CC 99-C-0143 were fludarabine naïve) and immune depletion prior to transplantation. We anticipate that the proposed increases in dose intensity (including the addition of rituximab) will not be associated with a significant increase in toxicity over what was seen with EPOCH-F in CC 99-C-0143. However, as a precaution our study design will allow for a reduction in dose intensity of the EPOCH-F/R regimen if specified toxicity criteria are met (Section 3.3.6).

For patients with CD20+ malignancies, adding the monoclonal antibody rituximab to the regimen is expected to boost the rate of response to EPOCH-F without significantly increasing the regimen's toxicity. Such an effect has been demonstrated in other studies where rituximab was administered with EPOCH or CHOP. In a phase II study, Wilson and colleagues administered dose-adjusted EPOCH plus rituximab to 38 patients with previously untreated aggressive or relapsed/resistant CD20+ B-cell lymphomas. An interim report indicates an 85% complete response rate among 34 evaluable patients; further, no patient in remission had progressed, with a median follow-up of 12 months. Hematologic toxicities were consistent with the dose-adjusted paradigm employed with

the EPOCH regimen, and non-hematologic toxicities were infrequent [22]. Similarly, Vose and colleagues conducted a phase II trial of CHOP plus rituximab in 33 previously untreated patients with aggressive B-cell non-Hodgkin's lymphomas. The overall response rate was 94%, with complete responses in 61%. After a median observation time of 26 months from remission, 29 of 31 patients who achieved CR or PR remained in remission. Observed toxicities were similar to those seen in studies of CHOP alone [23]. In a randomized study of 399 elderly patients with diffuse large B-cell lymphoma, complete responses were seen in 76% of patients receiving CHOP plus rituximab, versus 63% of patients treated with CHOP. Importantly, CHOP plus rituximab improved event-free and overall survival compared with CHOP alone, with no clinically significant difference in toxicity between the two arms [24].

Rituximab has been used as part of a reduced-intensity transplant conditioning regimen, with no adverse effect on hematopoietic recovery and engraftment and no reported impact on GVHD [25]. Ratanatharathorn and colleagues used rituximab to treat a patient with chronic GVHD and refractory immune thrombocytopenic purpura (ITP), leading to improvement in the patient's ITP and allowing the discontinuation of immunosuppression without progression of her chronic GVHD [26]. Apart from this highly anecdotal report, no published clinical data suggest that rituximab has activity in the prevention or treatment of GVHD; moreover, our current understanding of the pathophysiology of acute GVHD does not support a putative role for B-lymphocytes, so there is no rational basis for using rituximab to prevent or modulate GVHD. In this protocol, patients will receive rituximab no closer than four weeks before undergoing allogeneic HSCT, reducing the likelihood of an effect on donor B-cells after transplantation. For these reasons, we do not anticipate that combining rituximab with the EPOCH-F induction regimen (as EPOCH-F/R) will influence the primary GVHD endpoints of the current study. However, we will analyze for an effect of rituximab administration on hematopoietic recovery, donor/recipient chimerism, and the development of acute GVHD.

1.2.8 *Cyclosporine/Methotrexate as Graft-Versus-Host Disease Prophylaxis:* Although the combination of cyclosporine and methotrexate represents the *de facto* "gold standard" for acute GVHD prophylaxis in allogeneic HSCT, the use of cyclosporine monotherapy in CC 99-C-0143 was based partly upon the early belief that the incidence of GVHD might be lower following a reduced-intensity conditioning regimen [27]. In addition, there were concerns that more aggressive GVHD prophylaxis might compromise engraftment of donor stem cells, as the approach in CC 99-C-0143 had not previously been validated. With single-agent cyclosporine, 12 of the first 19 patients in CC 99-C-0143 developed grades II – IV acute GVHD; these patients did not receive donor Th2 cells. In six of these

patients, acute GVHD was severe (grades III-IV); two deaths were attributed to GVHD, while 4 additional patients died with progressive disease and grade II or III acute GVHD. Another 3 patients died of progressive disease with absent or grade I acute GVHD. These observations suggest that cyclosporine monotherapy is inadequate immunosuppression in the face of relatively rapid and complete engraftment of donor lymphoid cells.

Other studies of reduced-intensity allogeneic HSCT have also shown acute GVHD to contribute significantly to morbidity and mortality. In an attempt to improve this clinical problem, this protocol will evaluate dual-agent GVHD prophylaxis with cyclosporine and methotrexate as a potential strategy for preventing GVHD after reduced-intensity allogeneic HSCT. This approach differs from our other strategy for modulating GVHD, combining cyclosporine and donor Th2 cells. These two strategies (addition of methotrexate versus addition of donor Th2 cells) may have differential effects not only on GVHD modulation, but also on engraftment and anti-tumor effects. To this extent, after establishing the cyclosporine and methotrexate approach in this protocol, it will likely be beneficial to compare these two approaches in a randomized study.

In the ongoing study involving Th2 cells, 23 patients have received allogeneic HSCT and Th2 cells. Each of these patients achieved rapid full donor chimerism, with median day 14 donor chimerism of 100% (range, 85 to 100). Recovery of hematopoiesis was rapid, with a median neutrophil recovery at day 9 post-SCT (range, 7 to 13 days) and median platelet recovery at day 11.5 post-SCT (range, 8 to 12 days). Immune reconstitution research data indicates that Th2 cells promote donor T cell expansion and donor T cell cytokine secretion following HSCT. Acute GVHD severity in Th2 recipients has been Grade 0 (9/23 cases), Grade II (7/23 cases), and Grade III to IV (7/23 cases); these proportions are similar to those receiving allogeneic HSCT without Th2 cells. Significant anti-tumor responses have been observed in Th2 recipients. Of 22 evaluable patients after HSCT, 1/22 patients had a minor response (25 to 50% tumor reduction), 6/22 patients entered into a PR, and 15/22 patients entered into a CR. This high response rate, occurring in a patient population demonstrated to be chemotherapy refractory, suggests that allogeneic HSCT with Th2 cells is associated with a potent anti-tumor effect. Overall, seven of 23 Th2 recipients have died post-SCT. Three of these cases were associated with progressive disease, whereas 4 cases were not associated with progressive disease and were primarily related to transplant-related toxicity, GVHD, or immune suppression from GVHD therapy. Sixteen of 23 recipients are currently alive at a median of 156 days post-SCT (range, 47 to 606 days). This data from Th2 recipients, when combined with future data generated from this protocol evaluating cyclosporine and methotrexate GVHD prophylaxis, will be used to

determine if a randomized study comparing these two approaches might be warranted.

Studies comparing methotrexate with cyclosporine as single agent prophylaxis for GVHD showed the two agents to be roughly equivalent in their efficacy [28]. The methotrexate regimen administered in these studies was 15 mg/m^2 IV on day 1, then 10 mg/m^2 IV on days 3, 6, 11, and every 7 or 14 days thereafter until day 102 after transplantation. Combination regimens with methotrexate plus cyclosporine or FK506 (tacrolimus) have been used successfully for GVHD prophylaxis in both myeloablative and reduced-intensity transplantation settings. Storb and colleagues first compared the combination of cyclosporine and methotrexate with cyclosporine monotherapy, showing in a randomized trial that the combination reduced GVHD and improved survival [29, 30]. In this study, a short course of methotrexate was administered at doses of 15 mg/m^2 IV on day 1 and 10 mg/m^2 IV on days 3, 6, and 11 after transplantation; however, acute toxicity (mainly mucositis and renal insufficiency) still prevented completion of the full course of methotrexate in 42% of patients. Modified combination regimens with reduced methotrexate doses and/or shortened courses have subsequently been studied, although not in a prospective, randomized fashion; these appear to decrease the acute toxicity of methotrexate administration while preserving the benefits with respect to GVHD [31-34]. A commonly used regimen that has emerged from these studies is "mini-dose" methotrexate, given as 5 mg/m^2 on days 1, 3, 6, and 11.

In the setting of reduced-intensity allogeneic HSCT, studies of combination regimens (cyclosporine or tacrolimus, with methotrexate) have not been associated with high rates of graft rejection. GVHD outcomes in these studies have been inconsistent, with rates of grades III-IV acute GVHD ranging from 5% to 29%; this variability may reflect differences in transplant conditioning regimens and other risk factors [25, 35-37]. It therefore appears that dual-agent GVHD prophylaxis regimens containing methotrexate do not inhibit engraftment in the reduced-intensity transplant setting and may provide better prophylaxis of acute GVHD than cyclosporine monotherapy. Considering these factors, we intend to use cyclosporine with a mini-dose methotrexate regimen for GVHD prophylaxis in the current protocol. The evaluation of this regimen in the current study will serve as a platform for further evaluation of donor Th2 cells in a subsequent randomized study (see Section 1.2.2), with cyclosporine and methotrexate as a control arm versus cyclosporine and Th2 cells.

We believe (and other investigators have demonstrated) that the conversion to full donor chimerism after transplantation is a prerequisite for developing a GVT response [38]. Although engraftment kinetics and

the development of a GVT effect could be altered by the use of cyclosporine and methotrexate in the current study, the robust engraftment seen in CC 99-C-0143, with achievement of early donor chimerism in nearly all patients transplanted (Section 1.2.5), provides reassurance in the face of such concerns. As a secondary endpoint, we will evaluate the effect of the combination GVHD prophylaxis regimen upon hematologic recovery and chimerism in this study.

1.2.9 *Peripheral CD4 Lymphocyte Counts as a Surrogate Marker for Immune Depletion before Reduced-Intensity Allogeneic HSCT:* The robust engraftment results observed on CC 99-C-0143 support the principle of sequential immune depletion with an induction regimen such as EPOCH-F/R. Engraftment occurred similarly in patients across a range of CD4 counts prior to transplantation, suggesting that pre-transplant immune depletion was adequate within that range. Because no episodes of graft rejection were observed, it is not possible to derive from our results a "threshold level" of CD4 lymphopenia that distinguishes between patients likely to engraft versus those likely to experience graft rejection. In addition, the CD4 count may underestimate the level of immunosuppression after chemotherapy, as there may be functional abnormalities in residual CD4+ T cells after chemotherapy that reduce the likelihood of a host-versus-graft response and graft failure [39]. Nevertheless, it is likely that some patients in CC 99-C-0143 received induction cycles of EPOCH-F beyond the point at which engraftment would have occurred and without further benefit in terms of disease response. For such patients (i.e., those in remission or with stable disease), additional cycles of EPOCH-F – administered with the goal of achieving a CD4 count less than 50/μl – would have provided no clinical benefit and may have resulted in added toxicity.

It is plausible that a higher CD4 level (e.g., 200/μl or more) would be sufficient immune depletion to ensure engraftment; 90% of patients in CC 99-C-0143 had a CD4 count below 200/μl after EPOCH-F, and this level of CD4 lymphopenia corresponds to a demonstrable reduction in cell-mediated immunity in other clinical settings (e.g., human immunodeficiency virus infection). However, the addition of methotrexate to our GVHD prophylaxis regimen (see above) could increase the frequency of mixed chimerism after transplantation, as well as the risk of graft rejection. In the setting of T-cell-depleted allogeneic HSCT, the CD4 level before reduced-intensity conditioning correlates inversely with levels of donor lymphoid chimerism after transplantation (M. Bishop, personal communication). Balancing these considerations with the desire to minimize toxicity from the induction regimen, we have empirically chosen a CD4 level below 100 cells/μl after induction therapy with EPOCH-F/R as a target for the current study. Failure to reach this target after 3 cycles of induction therapy or due to disease progression will

not, however, prevent patients from proceeding to transplantation. As in CC 99-C-0143, we will correlate the CD4 count after EPOCH-F/R with engraftment outcomes following transplantation, in a further effort to validate the CD4+ T cell count as a surrogate marker for immune depletion before transplantation.

1.2.10 *FDG-PET versus CT Scanning in Hodgkin's and Non-Hodgkin's Lymphoma:* After allogeneic HSCT for Hodgkin's disease and non-Hodgkin's lymphoma, traditional imaging modalities such as computed tomography (CT) scanning are suboptimal for detecting residual or relapsed malignancy post-transplant. Restaging of patients with a history of bulky disease using CT scanning typically does not differentiate between a residual non-malignant mass (CR/unconfirmed; CRu [40]) or persistent malignant disease. Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) scanning, because it measures biologically active tumor, may allow one to distinguish patients with CRu from those with persistent malignancy [41]. In addition, because of its greater sensitivity compared with CT scanning, the use of FDG-PET may permit earlier detection of malignant disease progression post-transplant. The ability to distinguish CRu from persistent disease and to detect early relapse may improve transplant therapy, as patients with disease persistence or progression may benefit from transplant modulation such as early withdrawal of immune suppression or administration of donor lymphocytes.

In this study, we will use both FDG-PET and CT scanning in the staging of patients with Hodgkin's and non-Hodgkin's lymphoma. These studies will be obtained at study entry; before admission for allogeneic SCT (after induction chemotherapy with EPOCH-F/R); and on days 28 and 100 after allogeneic HSCT. Combined modality imaging will assist in distinguishing between CRu (PET negative) and persisent malignancy (PET positive) after transplantation. In cases where patients enter into CR after allogeneic HSCT, FDG-PET and CT will be obtained at 6 and 12 months post-transplant to facilitate the earlier detection of disease relapse.

1.2.11 *Interleukin-7 and Allogeneic HSCT:* Immune reconstitution following allogeneic HSCT is a prolonged process, influenced strongly by the age and corresponding thymic function of the transplant recipient, as well as the presence or absence of GVHD. Natural killer cells recover to normal levels soon after immune-depleting therapy, followed closely by B-cells within 1 to 3 months. Peripheral blood CD8+ T cells generally return to normal levels within 3 to 6 months, while CD4+ T-cell lymphopenia may persist for 24 months or longer [42]. Interleukin-7 (IL-7) is a major cytokine regulator of B-cell development and T-lymphocyte homeostasis [43]. Several recent reports indicate that circulating levels of IL-7 increase in response to lymphocyte or T-cell depletion in the setting of bone marrow transplantation, HIV infection, and cytotoxic chemotherapy

[44-46]. This homeostatic response appears to correlate most strongly with circulating numbers of CD4+ T cells, and it leads to increased thymic output and in particular to the thymic-independent peripheral expansion of mature T cells. In animal models of bone marrow transplantation or cytotoxic chemotherapy, administration of IL-7 increases the rate of T-cell immune reconstitution [47-49]. In one murine allogeneic BMT model, this effect was not associated with exacerbation of GVHD [49]. However, Sinha recently reported the finding that IL-7 significantly potentiates GVHD after allogeneic BMT in mice, an effect that increased with the number of T cells in the allograft [50].

In CC 99-C-0143, our group has studied the kinetics of CD4+ T-cell depletion in the setting of sequential immune depletion and reduced-intensity allogeneic HSCT as outlined above (Section 1.2.5). Other work by investigators in our branch has documented the variable recovery of CD4+ T cells following cytotoxic chemotherapy [39]. However, no published studies have specifically correlated serum IL-7 levels with lymphocyte subpopulations prior to transplantation and during immune recovery afterwards. We will include such an analysis as a secondary endpoint of this study, hypothesizing that serum IL-7 levels will correlate strongly with changes in lymphocyte subpopulations, especially CD4+ T cells, during immune-depleting chemotherapy and after reduced-intensity transplantation. Additionally, we will assess the relationship between IL-7 levels and the development of clinical GVHD, as this has not been determined previously.

1.2.12 *Interleukin-15 and Allogeneic HSCT:* A second cytokine that could play a key role in lymphocyte recovery in the post-transplant period is interleukin 15 (IL-15), an important cytokine in the differentiation, maintenance and expansion of NK and CD8+ T cells [51]. IL-15 is constitutively and ubiquitously produced by antigen presenting cells [52]. Its production, moreover, is upregulated by a variety of stimuli that evoke an inflammatory response [53]. Kumaki has reported that serum levels of IL-15 were elevated following transplant conditioning with myeloablative chemotherapy and total body irradiation [54]; furthermore, this elevation was prolonged in patients who developed acute GVHD. This work, however, did not assess any correlation between IL-15 levels and lymphocyte recovery in these patients.

IL-15 enhances CD8 production by thymopoiesis [55], but its main role appears to be in the periphery. IL-15, like IL-7, can stimulate homeostatic expansion of CD8+ T cells, particularly memory CD8+ T cells [56, 57]. Evidence from IL-15 and IL-15Rα knock-out mice demonstrates that IL-15 is critical to maintaining CD8+ T cell memory populations [58, 59]. IL-15 also stabilizes activated human T cells, reducing their level of apoptosis [60]. Furthermore, IL-15 has been reported to reduce the high

level of spontaneous apoptosis observed in post-transplant lymphocytes cultured in vitro [61]. There is a marked dichotomy between the high frequency of CD8+ T cell apoptosis in vitro and the elevated CD8 populations maintained in most post-transplant recipients in vivo. The anti-apoptotic efficacy of IL-15 *in vitro* would support the hypothesis that *in vivo* IL-15 production may be maintaining CD8+ T cell viability.

IL-15 may also play a role in the functional capacities of post-transplant CD8+ T cells. IL-15 upregulates the expression of co-stimulatory receptors, such as NKG2D, on activated CD8+ T cells [62]. These receptors may be important in the cytotoxic responses to stress and to viral infection, both relevant in the post-transplant period [63]. The role of these receptors in the allogeneic responses of GVHD has not been studied.

IL-15 is also the central cytokine in the differentiation, expansion and activation of NK cells [64]. NK cells are the earliest of the lymphocyte populations to recover to pre-treatment levels following transplantation or chemotherapy [39]. Cytokines such as IL-4 and IFNγ produced by NK cells may play a critical role in initiating type I and type II T cell responses [64]. Further, NK cells may be critical cytotoxic effectors in the graft-versus-leukemia response, without corresponding GVHD [65, 66]. IL-15 is the central cytokine supporting the differentiation of both the cytokine producing and the cytotoxic subpopulations of NK cells [59, 64, 67]. As is the case with CD8+ T cells, the expression levels of NK receptors involved in activating or inhibiting cytotoxicity may also be altered by IL-15. The repertoire of such receptors expressed by NK cells following transplantation may affect their function in GVHD or GVL [65]. The role of IL-15 in the post transplant recovery of NK cells and in the repertoire of receptors expressed by these cells, however, has never been explored.

These observations suggest an important physiologic role for IL-15 in the recovery and maintenance of NK and CD8+ T cells after immune depletion related to cytotoxic chemotherapy or HSCT, with potential implications for tumor-specific immune responses and immune reconstitution following transplantation. The additional observation that IL-15 levels remained elevated in patients with acute GVHD raises the possibility of further interactions between IL-15 and lymphocyte subpopulations in the pathogenesis of GVHD. We therefore intend to correlate serum levels of IL-15 with circulating CD8+ T cell and NK cell populations, during sequential immune depletion and after reduced-intensity allogeneic HSCT during immune recovery. The purpose of this analysis will be to explore potential relationships among IL-15, lymphocyte subpopulations, and the development of GVHD.

1.3 Summary: The strategy proposed in this protocol has several goals. First, we will pilot an intensified induction chemotherapy regimen, EPOCH-F/R, by building on the EPOCH-F component used in CC 99-C-0143 and adding rituximab for patients with CD20+ malignancies, in order to improve disease control and accelerate immune depletion to our CD4 target level of less than 100 cells/μl. Second, and most importantly, we will employ a dual-agent regimen of cyclosporine and methotrexate for GVHD prophylaxis, with the objective of improving upon the incidence of acute GVHD observed in CC 99-C-0143. Third, we will correlate serum levels of IL-7 and IL-15 with changes in circulating lymphocyte subpopulations during sequential immune depletion with EPOCH-F/R and after reduced-intensity allogeneic HSCT, to contribute to our understanding of the immunologic mechanisms that underlie immune ablation, activation and reconstitution in the transplant setting. The stated goals will permit our group to refine our approach to reduced-intensity allogeneic HSCT for refractory and relapsed hematologic malignancies, setting the stage for subsequent research efforts such as a randomized trial of donor Th2 cells.

2.0 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:

Disease	Disease Status	Age
Chronic Lymphocytic Leukemia	a) Relapse post-fludarabine b) Non-CR after salvage regimen	18 to 75
Hodgkin's and Non-Hodgkin's Lymphoma (all types, including Mantle Cell Lymphoma)	a) Primary treatment failure b) Relapse after autologous SCT c) Hepatosplenic gamma/delta T cell lymphoma	18 to 75
Multiple Myeloma	a) Primary treatment failure b) Relapse after autologous SCT c) Non-CR after salvage regimen	18 to 75
Acute Myelogenous Leukemia	a) In Complete Remission #1, with high-risk cytogenetics [abnormalities other than t(8;21), t(15;17), or inv(16)] b) In Complete Remission #2 or greater	18 to 75
Acute Lymphocytic Leukemia	a) In Complete Remission #1, with high-risk cytogenetics [e.g., t(4;11), t(1;19), t(8;14); t(9;22) or *bcr-abl* rearrangement] b) In Complete Remission #2 or greater	18 to 75
Myelodysplastic Syndrome	a) RAEB b) RAEB-T (if blasts are < 10% in marrow and blood after induction chemotherapy)	18 to 75
Myeloproliferative disorders	a) Idiopathic myelofibrosis b) Polycythemia vera c) Essential thrombocytosis d) Chronic myelomonocytic leukemia	18 to 75
Chronic Myelogenous Leukemia	a) Chronic phase CML b) Accelerated phase CML	50 to 75
	c) Not eligible for myeloablative allogeneic HSCT	18 to 50

b) Patients 18 - 75 years of age. Patients older than 75 years of age will be considered on an individual basis [68].
c) Consenting first degree relative matched at 6/6 HLA antigens (A, B, and DR).
d) Patient or legal guardian must be able to give informed consent.
e) All previous cytotoxic chemotherapy must be completed at least 2 weeks prior to study entry. Any grade 3 or 4 non-hematologic toxicity of any previous therapy must have resolved to grade 2 or less, unless specified elsewhere in Section 2.1.
f) ECOG performance status equal to 0, 1, or 2, and Karnofsky performance status greater than or equal to 60%.
g) Life expectancy of at least 3 months.
h) Patients with acute leukemia must be in hematologic remission, defined as less than 5% blasts present in blood or bone marrow.
i) Left ventricular ejection fraction > 45% by either MUGA or 2-D echo, obtained within 28 days of enrollment. The cumulative dose of doxorubicin received by patients will not be considered, as the cardiac ejection fraction appears to indicate the safe cumulative doxorubicin dose in the setting of EPOCH-based chemotherapy [18].
j) DLCO > 50% of the expected value when corrected for Hb [69], obtained within 28 days of enrollment.
k) Creatinine $\leq$ 1.5 mg/dl and creatinine clearance $\geq$ 50 ml/min/1.73 m^2.
l) Serum total bilirubin less than 2.5 mg/dl, and serum ALT and AST values less than or equal to 2.5 times the 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. If these values do not normalize during induction chemotherapy, such patients will not be eligible for the transplant phase of the protocol, and will thus be taken off study.
m) Minimum absolute neutrophil count of 1,000 cells/µl and minimum platelet count (without transfusion) of 20,000/mm^3. Values below these levels may be accepted at the discretion of the PI or LAI, if thought to be due to bone marrow involvement by malignancy.

2.1.2 Inclusion Criteria – Donor:

a) First-degree relative with genotypic identity at 6/6 HLA loci (HLA- A, B, and DR).
b) Ability to give informed consent. For donors under 18 years of age, the donor must complete an assent form, and the donor's legal guardian must complete an informed consent form.
c) Age 12 – 75 years. As the potential cerebrovascular and cardiac complications may potentially increase with age, age 75 has been chosen arbitrarily as the upper age limit. However, if it is determined after initial accrual of patients in this upper age range that this procedure is relatively safe, the age range may be extended.

d) Adequate venous access for peripheral apheresis, or consent to use a temporary central venous catheter for apheresis.
e) Donors must be HIV negative, hepatitis B surface antigen negative, and hepatitis C antibody negative. This is to prevent the possible transmission of these infections to the recipient.
f) A donor who is lactating must substitute formula feeding for her infant during the period of cytokine administration. Filgrastim may be secreted in human milk, although its bioavailability from this source is not known [70]. Limited clinical data suggest that short-term administration of filgrastim or sargramostim to neonates is not associated with adverse outcomes [71].

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. There is theoretical concern that the degree of immune suppression associated with the treatment may result in progression of HIV infection.
d) Chronic active hepatitis B. Patients may be hepatitis B core antibody positive but must be surface antigen negative and without evidence of active infection.
e) Hepatitis C infection.
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 (as determined by principal investigator or study chairman).

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, or severe heart disease. Individuals with symptomatic angina will be considered to have severe heart disease and will not be eligible to be a donor.
c) No other medical contraindications to stem cell donation (i.e. severe atherosclerosis, autoimmune disease, cerebrovascular accident, prior malignancy) [72]. Patients with a history of coronary artery bypass grafting or angioplasty will receive a cardiology evaluation and be considered on a case-by-case basis. Persons with a history of non-hematologic malignancy must have undergone potentially curative

therapy for that malignancy and (1) have had no evidence of that disease for 5 years, and/or (2) be deemed at low risk for recurrence (less than or equal to 20% at 5 years). Such persons will be considered eligible for stem cell donation at the discretion of the principal investigator, who will evaluate the possible benefit to the potential transplant recipient and the risk of disease transmission in consultation with Department of Transfusion Medicine staff. Prospective donors with a history of non-hematologic malignancy who have received potentially curative therapy and are in remission, but whose estimated risk of recurrence is greater than 20% at 5 years, will be considered on an individual basis in consultation with the NCI IRB. Any prospective transplant recipient whose donor has a history of malignancy will be counseled about the theoretical risk of transmission of cancer from the donor to the recipient.

d) Donors must not be pregnant. The effects of cytokine administration on a fetus are unknown. Donors of childbearing potential must use an effective method of contraception.
e) Anemia (Hb less than 11 gm/dl) or thrombocytopenia (platelets less than 100,000 per μl).

2.2 Research Eligibility Evaluation:

2.2.1 The following clinical, laboratory and radiologic assessments must be performed in the patient (recipient) within 28 days before the initiation of induction chemotherapy (Section 3.3) to determine disease status:

a) Complete medical history and physical examination.
b) Nutritional assessment (initial consult).
c) Dental consultation to assess need for teeth cleaning or removal.
d) Social work consultation.
e) Typing for HLA-A, -B, and –DR.
f) Antibody screen for hepatitis A, B, and C; HIV, HTLV-I/II, CMV, adenovirus, EBV, HSV, Toxoplasma, and syphilis.
g) PPD test (if considered to be in a high-risk group).
h) PT, PTT, and ABO typing.
i) Urinalysis.

Items (j) through (l) should be obtained within 48 hours before starting induction chemotherapy.

j) CBC with differential.
k) Acute care panel, hepatic panel, and mineral panel.
l) Urine βHCG in women of childbearing potential.
m) Unilateral bone marrow aspirate and biopsy. Flow cytometry, cytogenetics, and molecular testing via polymerase chain reaction (PCR) should be performed on marrow aspirates as deemed clinically appropriate for specific diseases.
n) Chest radiograph.
o) Electrocardiogram.

p) CT scans of chest, abdomen, and pelvis (neck included if measurable disease is present).
q) CT or MRI of the head.
r) PET scan for recipients with non-Hodgkin's lymphoma or Hodgkin's lymphoma [41].
s) Skeletal survey (for multiple myeloma patients only).
t) Other specific serum or urine diagnostic studies clinically appropriate for the evaluation of recipient's malignancy (e.g., SPEP and UPEP for multiple myeloma).
u) All biopsy specimens will be reviewed by the Laboratory of Pathology, CCR, NCI, for confirmation of the histologic diagnosis prior to initiation of therapy.
v) Biopsy material or blood samples from patients with B-cell malignancies will be tested for CD20 expression, to confirm eligibility for rituximab with induction chemotherapy. CD20 expression will be determined by immunohistochemistry or flow cytometry in the Laboratory of Pathology, CCR, NCI.
w) Flow cytometric analysis of peripheral blood for baseline measurement of CD3, CD4, CD8, and CD56 positive lymphocyte populations.
x) Baseline determination of serum IL-7 and IL-15 levels, for correlation with NK and T-cell subpopulations (above).
y) PCR test of DNA mini-satellite regions for future determination of chimerism.

2.2.2 The following clinical, laboratory and radiologic assessments must be performed in the donor within 28 days before study enrollment:
a) Complete medical history and physical examination.
b) Social work consultation.
c) Typing for HLA-A, -B, and –DR.
d) Antibody screen for hepatitis A, B, and C; HIV, HTLV-I/II, CMV, adenovirus, EBV, HSV, Toxoplasma, and syphilis.
e) CBC with differential, PT, PTT, and ABO typing.
f) Acute care panel, hepatic panel, and mineral panel.
g) Urinalysis.
h) Urine βHCG in women of childbearing potential.
i) Chest radiograph.
j) Electrocardiogram.
k) PCR test of DNA mini-satellite regions for future determination of chimerism.

2.3 Patient Registration:

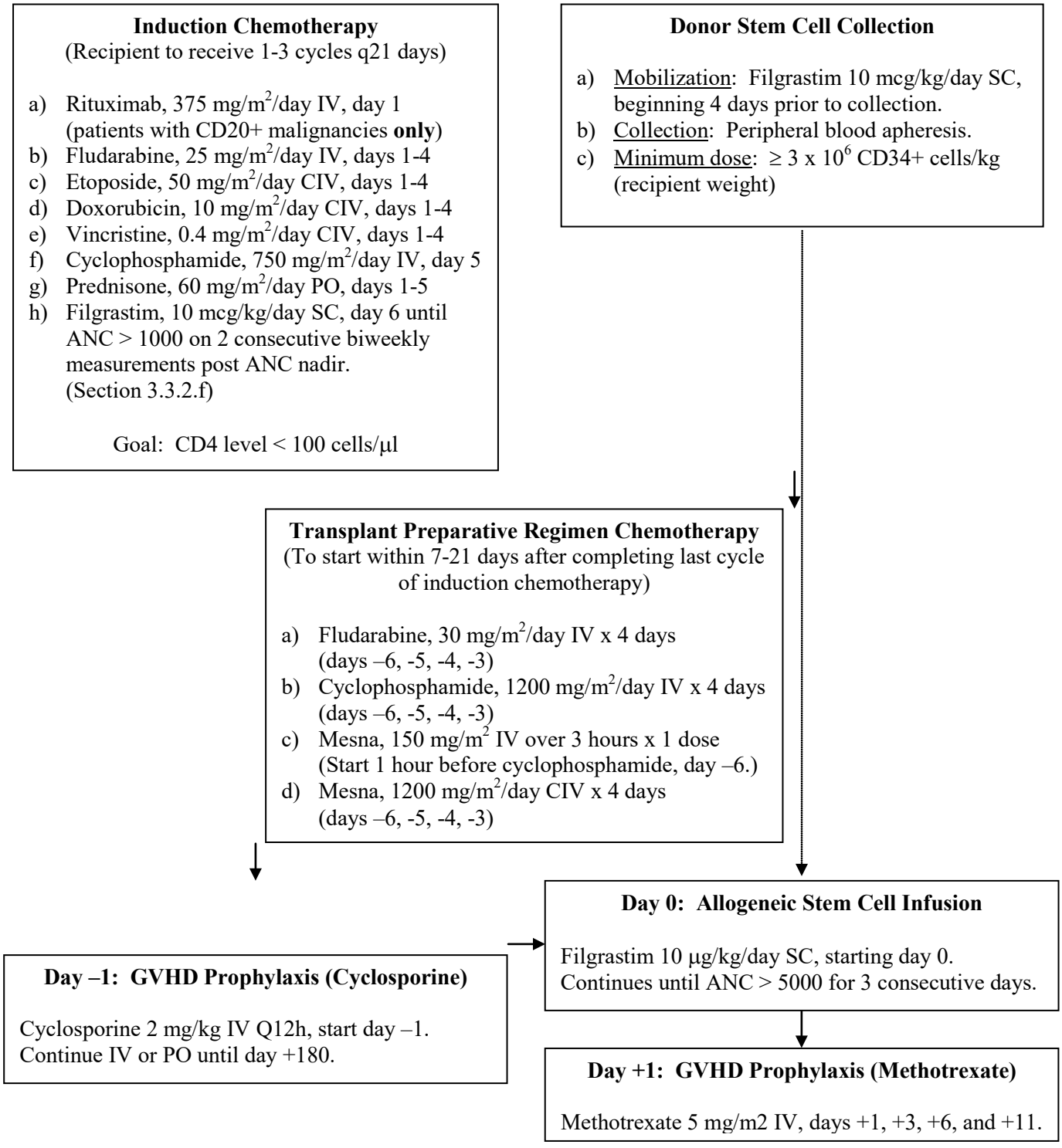
2.3.1 The patient's entry date on protocol is considered to be the day that consent forms have been signed by both the donor and the recipient. The treatment start date is considered to be the day the recipient begins his/her initial induction cycle (Section 3.5).

2.3.2 Authorized staff must register an eligible candidate with ORKAND no later than 24 hours after both the 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 on which an eligible donor signs consent may precede the patient's date of consent by up to four weeks, in some cases. 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 must be completed and faxed to ORKAND at 301-480-0757. After confirmation of eligibility at ORKAND, ORKAND staff will call Pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any protocol pharmacotherapy. For registration, authorized staff should call 301-402-1732 between the hours of 8:30 a.m. and 5:00 p.m., Monday through Friday. A recorder is available during non-working hours.

3.0 Study Implementation

3.1 Study Design:



3.2 Stem Cell Mobilization and Collection**:**

3.2.1 Donors will receive filgrastim for stem cell mobilization, at a dose of 10 μg/kg/day by subcutaneous injection. The filgrastim will be given at approximately 7:00 am each day.

3.2.2 Apheresis will start on the fifth day of filgrastim administration. 15-25 liters will be processed daily. Apheresis and filgrastim administration will continue daily for up to 3 consecutive days until the target cell dose of $\geq 4 \times 10^6$ CD34+ cells/kg-recipient weight is obtained. If the minimum cell dose of 3×10^6 CD34+ cells/kg-recipient weight is obtained during the first mobilization cycle, no further collection will be scheduled, and the patient will be eligible for transplantation. Donors who do not reach the minimum number of 3×10^6 CD34+ cells/kg-recipient weight after three collections will discontinue filgrastim for a minimum of three weeks, after which the mobilization and collection will be attempted again. If the minimum number of cells has not been reached at this time, the patient will be removed from the trial.

3.2.3 Cells will be processed, cryopreserved, and stored in liquid nitrogen until the day of transplant. If an ABO incompatibility exists between the donor and patient, the donor erythrocytes will be removed from the graft in the cell processing laboratory, according to standard procedures.

3.3 Induction Chemotherapy:

3.3.1 After enrollment, chemotherapy will be initiated as an outpatient. Adequate central venous access is required for all patients undergoing transplantation on this protocol.

3.3.2 EPOCH-Fludarabine/Rituximab (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 per day IV Infusion over 30 minutes, Daily for 4 days	Days 1, 2, 3, 4
Etoposide	50 mg/m^2 per day continuous IV Infusion over 24 hours, Daily for 4 days	Days 1, 2, 3, 4
Doxorubicin	10 mg/m^2 per day continuous IV Infusion over 24 hours, Daily for 4 days	Days 1, 2, 3, 4
Vincristine	0.4 mg/m^2 per day continuous IV Infusion over 24 hours, Daily for 4 days	Days 1, 2, 3, 4
Cyclophosphamide	750 mg/m^2 IV Infusion over 30 minutes	Day 5 only
Prednisone	60 mg/m^2 per day orally, daily for 5 days	Days 1, 2, 3, 4, 5

Filgrastim	10 μg/kg per day subcutaneously	Daily from day 6 until ANC > 1000/μl x 2 following nadir (Section 3.3.2.f)

a) Only patients with CD20+ B-cell malignancies will receive rituximab as part of the induction chemotherapy regimen. The initial infusion should begin at a rate of 50 mg/hr; if no hypersensitivity reaction is observed after the first 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 for subsequent administration. Premedication with acetaminophen 650 mg PO and diphenhydramine 50 mg IV will be administered 30 to 60 minutes prior to the beginning of each rituximab infusion. Rituximab should be infused prior to the administration of fludarabine and the start of infusional EPOCH chemotherapy on the first day of each cycle.

b) Fludarabine will be given immediately after the rituximab infusion is completed on day 1. If venous access permits, fludarabine may be given concurrently with the start of infusional EPOCH chemotherapy on days 1 through 4 of each cycle (preferred); otherwise, fludarabine will be given before the start of EPOCH infusional agents on days 1-4.

c) Etoposide, doxorubicin, and vincristine will be prepared and administered according to NIH Clinical Center Pharmacy standard guidelines (section 8.6).

d) The total vincristine dose per cycle will not be capped, even if it exceeds 2.0 mg over four days.

e) Cyclophosphamide will be administered on day 5 of each cycle immediately after the prior day's infusion is completed. Hydration with 1 liter of 0.9% sodium chloride is recommended to begin one hour prior to cyclophosphamide, infused over two hours.

f) Because immune depletion with EPOCH-F/R is a secondary endpoint of this study, patients should receive a consistent steroid regimen with each cycle of induction chemotherapy. Therefore, steroids will not be used as an anti-emetic during EPOCH-F/R chemotherapy.

g) Filgrastim will be initiated on day 6 at a dose of 10 μg/kg/day; it will be continued until the ANC is greater than 1000 cells/μl on two consecutive biweekly measurements following the ANC nadir, or greater than 5000 cells/μl on 1 biweekly measurement. Biweekly measurements are obtained at least 3 days apart (e.g., Monday and Thursday). Only biweekly CBC values will be used to determine the

duration of filgrastim administration during each cycle, even if additional CBC's are obtained.

3.3.3 Determination of Number of Cycles of Induction Chemotherapy:

a) Because a major goal of the induction chemotherapy is to establish severe host immune T cell depletion prior to the allogeneic HSCT, the number of induction chemotherapy cycles administered will be determined principally by the level of host T cell depletion achieved.

b) The CD4 count will be measured by FACS analysis 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). FACS analysis will be performed by William Kopp, Ph.D. at FCRF using a CLIA certified flow cytometry methodology to determine the percentage of circulating CD4+ cells. A simultaneous CBC will be drawn in order to calculate the absolute CD4 number.

c) If a patient has fewer than 100 CD4 cells/μl of blood on the final CD4 determination of the induction chemotherapy cycle, then that patient will receive the transplant preparative regimen. If there are $\geq$ 100 CD4 cells/μl, an additional cycle of induction chemotherapy will be administered. These criteria may be used for each induction chemotherapy cycle. A minimum of one cycle of induction chemotherapy will be administered. However, a maximum of three cycles of induction chemotherapy can be administered.

d) 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 before administration of the second cycle (e.g., for delay in neutrophil recovery to 1000 cells/μl, documented infection or other complication resulting from the induction chemotherapy regimen).

e) If a patient develops neutropenia of less than 500 cells/μl on 4 or more consecutive biweekly CBC's during any cycle of induction chemotherapy 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, even if the CD4 count is $\geq$ 100 cells/μl. Only biweekly CBC values (at least 3 days apart, e.g. Monday and Thursday) will be used to define the duration of neutropenia during each cycle, even if additional CBC's are obtained.

f) If the maximum of three cycles of induction chemotherapy has been administered, then patients will proceed to the transplant preparative regimen, even if the CD4 count is still $\geq$ 100 cells/μl.

g) Patients who develop progressive disease during the induction chemotherapy cycles will proceed to the transplant preparative regimen regardless of the CD4 count (see also Section 3.8.1.d).

3.3.4 Dose Adjustment for Cycles 2 and 3 (for individual patients)

a) Dose adjustments <u>above starting dose level</u> (level 1) consist of a 20% escalation in the daily doses of etoposide, doxorubicin, and cyclophosphamide.

b) 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 cycle, based upon consecutive biweekly CBC/differential (≥ 3 days apart). Only biweekly CBC values will be used to determine dose adjustment, even if additional CBC's are obtained.
d) Rules for dose adjustment are summarized in the following table:

3.3.5 Dose Reduction of EPOCH-F/R Induction Chemotherapy (for study population)

a) The following dose reduction rules will apply only to cycles of EPOCH-F/R that are administered at dose level 1 or lower (see section 3.3.4, above).
b) If three patients experience either: (1) grade IV hematologic toxicity (ANC less than 500 cells/μl) lasting for 4 or more consecutive biweekly measurements; or (2) any grade IV non-hematologic toxicity by the NCI Common Toxicity Criteria, then fludarabine will be reduced from four days to three days of administration, without changing the daily dose of 25 mg/m^2.
c) If, after such a change in the EPOCH-F/R regimen is made, three patients again experience: (1) grade IV hematologic toxicity (ANC less than 500 cells/μl) lasting for 4 or more consecutive biweekly measurements; or (2) any grade IV non-hematologic toxicity, then the cyclophosphamide will be given on day 5 with a reduction in dose from 750 mg/m^2 to 600 mg/m^2.

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	→	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.

d) If dose reductions in the EPOCH-F/R regimen are made as described in Sections 3.3.6.a and 3.3.6.b (above), and three patients again experience: (1) grade IV hematologic toxicity (ANC less than 500 cells/μl) lasting for 4 or more consecutive biweekly measurements; or (2) any grade IV non-hematologic toxicity, then the etoposide, doxorubicin, and vincristine will be reduced from four days to three days of administration, without a change in daily dose of these drugs. In the event of this dose reduction, the cyclophosphamide will be given on day 4 of the cycle at a dose of 600 mg/m^2, and filgrastim will start on day 5. The dose and schedule of prednisone administration will not change.

3.4 Transplant Conditioning Regimen

3.4.1 The patient may proceed to transplantation only if the minimum number of stem cells is collected (Section 3.2.2).

3.4.2 On day 22 after the start of the last cycle of induction chemotherapy, patients will be eligible to start the transplant conditioning regimen. Non-hematologic toxicities of induction chemotherapy must resolve to < grade 2 in order to proceed. If more than 14 days of additional recovery time is required for resolution of toxicity or other medical complications (for example, prolonged neutropenia or documented infection), PI approval is required prior to initiation of the transplant conditioning regimen.

3.4.3 Fludarabine/Cyclophosphamide/Mesna:

Transplant Conditioning Regimen

Drug	Dose	Days
Fludarabine	30 mg/m^2 per day IV infusion over 30 minutes, daily for 4 days	Transplant Days –6, -5, -4, -3
Mesna	150 mg/m^2 IV infusion over 3 hours, (start 1 hour before cyclophosphamide)	Transplant Day –6 only
Cyclophosphamide	1200 mg/m^2 per day IV infusion over 2 hours, daily for 4 days	Transplant Days –6, -5, -4, -3
Mesna	1200 mg/m^2 per day Continuous IV infusion, daily for 4 days	Transplant Days –6, -5, -4, -3

3.4.4 Hydration during the conditioning regimen:

a) Hydration will be initiated 12 hours prior to cyclophosphamide infusion (on day –7 of the transplant), consisting of 0.9% sodium chloride supplemented with 10 mEq/liter potassium chloride (KCl) at an initial rate of 100 ml/hour. For patients with poor oral intake, the rate of hydration may be increased as clinically indicated to meet fluid requirements. 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 saline infusion. If serum potassium is < 3.0, KCl concentration in the saline 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. In general, furosemide doses should be separated by at least a four-hour observation interval.

d) During hydration, if urine output is < 1.5 ml/kg/hour or if fluid intake exceeds urine output by greater than 500 ml during an 8-hour period, an additional 20 mg of furosemide will be administered.

e) Routine hematologic monitoring, cardiac monitoring, and urinalysis will be performed as deemed clinically necessary.

3.5 Transplant Procedure: Allogeneic Peripheral Blood Stem Cell Transplantation

3.5.1 On day 0, the patient will receive cryopreserved PBSC. The PBSC product will be administered intravenously immediately after thawing.

3.5.2 Diphenhydramine and/or meperidine are preferred for the treatment of acute DMSO toxicities (e.g., chills, myalgias); steroids should be avoided.

3.6 Graft-Versus-Host Disease Prophylaxis:

3.6.1 Cyclosporine (CsA) will be initiated on the day –1 before the transplant. CsA will be administered by IV infusion at 2 mg/kg/dose every 12 hours; infusion will be 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. The target range for serum cyclosporine levels will be 200–250 μg/ml. When the patient is able to take oral medications (typically two to three weeks after transplantation), cyclosporine will be converted to an equivalent oral dose. The total daily dose will be divided into two equal doses, one dose given approximately every 12 hours.

3.6.2 This dose of CsA will continue until day +100, and then will be tapered as long as the severity of GVHD is less than grade 2. Cyclosporine will be tapered by reducing the dose 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.

3.6.3 Methotrexate will be given at 5 mg/m^2 IV over 15 minutes on days +1, +3, +6, and +11. Each day's dose of methotrexate will not be administered until approved that day by the transplant attending after the transplant team has evaluated the patient. Doses will be withheld for the

development of grade III or IV mucositis or clinical evidence of veno-occlusive disease. Administration of the combination of trimethoprim and sulfamethoxazole (i.e., Bactrim®) or non-steroidal anti-inflammatory drugs is contraindicated during methotrexate administration. Methotrexate administration will be assessed on each planned day of administration, and dose adjustments will be based upon the following criteria:

Creatinine	Total Bilirubin	Methotrexate Dose
< 1.5 mg/dL	< 2.0 mg/dL	100%
1.6 – 2.0 mg/dL	2.1 – 3.0 mg/dL	75%
2.1-2.5 mg/dL	3.1 – 4.0 mg/dL	50%
> 2.5 mg/dL	> 4.0 mg/dL	Hold Dose

3.7 Treatment of Mixed Chimerism, Bone Marrow Aplasia, or Persistent/Progressive Disease Post-Transplant

3.7.1 Donor Lymphocyte Infusion

a) Patients with persistent or progressive malignancy post-HSCT, or mixed chimerism that does not improve after tapering or discontinuing immune suppression, will be eligible to receive donor lymphocytes (donor lymphocyte infusion, or "DLI") as a component of this study. DLI may be administered alone or after chemotherapy.

b) Donor lymphocytes will be collected by apheresis from the patient's stem cell donor, either in steady state (i.e., no donor therapy) or after filgrastim administration. The donor product will be divided into aliquots as follows:

- 1×10^6 $CD3^+$ T cells per kg of recipient weight (1 aliquot only, to use for treatment of mixed donor chimerism; not required if collection obtained for treatment of malignant disease)
- 1×10^7 $CD3^+$ T cells per kg of recipient weight (1 aliquot)
- 5×10^7 $CD3^+$ T cells per kg of recipient weight (remainder of collection)

c) DLI may be sequentially administered, with initial dosing at 1×10^6 $CD3^+$ T cells per kg of recipient weight for mixed chimerism, up to 1×10^7 $CD3^+$ T cells per kg for malignant disease. Subsequent doses may be escalated to 1×10^8 $CD3^+$ T cells per kg.

3.7.2 Secondary Infusion of $CD34^+$ Donor Stem Cells

a) Certain clinical situations may require additional donor stem cells without donor lymphocytes (e.g., bone marrow aplasia with established GVHD). In such cases, consenting donors will undergo repeat filgrastim mobilization and stem cell collection according to the procedure in section 3.2. However, the apheresis product will undergo positive selection of $CD34^+$ cells to achieve separation of donor stem cells and lymphocytes, according to standard DTM procedures using an FDA-approved device. This procedure ordinarily achieves a 4-log depletion of $CD3^+$ cells in the $CD34^+$ cell-selected product, relative to unmanipulated mobilized donor cells. The $CD34^+$ cell-selected

fraction will typically be administered in its entirety to the transplant recipient in these cases, with or without initial cryopreservation. The $CD3^+$ cell-enriched fraction may be divided into aliquots and cryopreserved for subsequent use if clinically indicated, as described in section 3.7.1(c).

3.7.3 Other Post-Transplant Therapy

a) In addition to DLI, persistent or progressive disease may be treated with any approved therapy thought to be in the best interest of the patient, such as chemotherapy, cytokines, or monoclonal antibodies. Alternatively, such patients may be offered therapy on other NCI protocols.

3.8 Interim Evaluation During Induction Chemotherapy:

3.8.1 The following studies will be obtained in the patient (recipient) at the end of each cycle of EPOCH-F/R induction chemotherapy:

a) Routine chemistry and hematology panels
b) For patients with lymphoma: computed tomography scans of chest/abdomen/pelvis (and neck, if clinically indicated)
c) For patients with multiple myeloma: 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.

3.9 Pre-Transplant Evaluation:

3.9.1 The following studies will be obtained in the patient (recipient) within 28 days before the initiation of the transplantation conditioning regimen to determine disease status:

a) Routine chemistry and hematology panels
b) Bone marrow aspiration and biopsy; flow cytometry, cytogenetics, and molecular studies as clinically appropriate
c) For patients with lymphoma or CLL: computed tomography scans of chest/abdomen/pelvis (and neck, if clinically indicated); FDG-PET scan.
d) For patients with multiple myeloma: 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; skeletal survey.

3.9.2 The following studies will be obtained in the patient (recipient) within four weeks before the initiation of the conditioning regimen:

a) Pulmonary function testing including DLCO
b) 2-D echo or MUGA scan
c) Serum creatinine, hepatic transaminases, total bilirubin
d) Urine β-HCG for women of childbearing potential.

3.10 Evaluation During Transplantation

3.10.1 The following studies will be obtained in the patient (recipient) during hospitalization for transplantation:

a) CBC twice daily; differential count daily

b) Acute care and mineral panels twice daily; hepatic panel and LDH daily
c) Type and screen every 3 days
d) Cyclosporine trough level twice weekly, starting day +1

3.11 Off Study Criteria:

3.11.1 The donor or recipient will be removed from protocol for any of the following reasons:

a) Inadequate stem cell collection (Section 3.2.2); this will result in the removal of both the donor and recipient.
b) Unacceptable toxicity ($\geq$ grade 3) for the donor.
c) Irreversible non-hematologic toxicity ($\geq$ grade 3) for the recipient while receiving induction chemotherapy (EPOCH-F/R).
d) The donor or recipient refuses to continue therapy.
e) 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.

3.11.2 Authorized physicians must notify ORKAND when a patient is taken off study.

3.12 Post Study Evaluation:

3.12.1 After completion of therapy the patient will be followed for potential complications related to allogeneic SCT. The patient will be followed at least twice weekly in the outpatient setting until at least Day +100 after discharge from the hospital.

3.12.2 The patient (recipient) will seen in follow-up at the Clinical Center to evaluate disease status and late problems related to allogeneic HSCT, at days +28 and +100; and at 6, 9, 12, 18, and 24 months post-transplant. At these times patients will have the following tests performed to determine clinical response:

a) Routine chemistry and hematologic panels.
b) Bone marrow aspiration and biopsy with flow cytometry, cytogenetics, and molecular studies as clinically appropriate.
c) For patients with lymphoma: computed tomography scans of chest/abdomen/pelvis (and neck, if clinically indicated). FDG-PET scan will be obtained 28 and 100 days post transplant in all patients with lymphoma. 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.
d) For patients with multiple myeloma: 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; skeletal survey.

3.12.3 Repeat pulmonary function testing will be performed at 100 days and 1 year post transplant. Repeat 2-D echocardiography or MUGA scan will be performed 100 days post transplant.

3.13 Immunologic Studies

3.13.1 Correlation between patient (recipient) T-cell subpopulations, serum IL-7 and IL-15 levels (Hakim/Gress & Fry laboratories):

a) Baseline evaluation of donor NK and T cells: 40 ml of heparinized whole blood (4 10cc green top tubes) prior to filgrastim mobilization; aliquot of donor apheresis product with ~50 x 10^6 mononuclear cells. Performed in Hakim/Gress lab (10/5A07).

b) Patient lymphocyte subpopulations: 60 ml heparinized whole blood (20 ml for FACS, 40 ml for RNA RT-PCR of NK receptors) to Hakim/Gress lab (10/5A07). Baseline before start of EPOCH-F/R induction chemotherapy; at end of each cycle of EPOCH-F/R; day 0 following administration of transplant preparative regimen; days +7, +14, +28, +60, +100; 6 months, 9 months, 12 months, 18 months, 24 months, and annually therafter.

c) Patient IL-7 and IL-15 levels: 10 ml whole blood (1 10cc red top tube), to be sent to Frederick for storage. IL-7 levels will be performed in Fry lab (10/13C210); IL-15 levels will be performed in Hakim/Gress lab (10/5A07). Baseline before start of EPOCH-F/R induction chemotherapy; at end of each cycle of EPOCH-F/R; day 0 following administration of transplant preparative regimen; days +7, +14, +28, +60, +100; 6 months, 9 months, 12 months, 18 months, 24 months, and annually therafter. Note: IL-7 levels will not include day +60 or time points after day 100.

d) Lymphocyte subpopulations, IL-7, and IL-15 levels may be obtained at other time points of interest (e.g., development of GVHD).

3.13.2 Correlation of lymphocyte cytokine expression patterns with GVHD in patients:

a) 40 ml of blood will be drawn in heparinized tubes each Thursday, starting from the second week post-SCT and extending until day 50 post-transplant. Samples will be sent to the Fowler Lab (10/12C210) to the attention of Jason Foley, 4-4537. These samples will be Ficolled, cultured overnight in media containing the golgi inhibitor monensin, and evaluated for monocyte surface markers and intracellular cytokine markers for CD14 monocyte production of IL-1 alpha, TNF alpha, and IL-15.

b) 50 ml of blood will be drawn into green top heparinized tubes on day 6 and day 7 post-transplant. Samples will be sent to the Fowler Lab (10/12C210) to the attention of Jason Foley, 4-4537. These samples will be Ficolled, and evaluated either fresh (day 7 sample) or after CD3, CD28 stimulation (day 6 sample) for T cell cytokine secretion profile. This assay will be by cytokine capture flow cytometry, with surface markers of CD4 and CD8 evaluated. Cytokine capture

staining of these populations will evaluate both T1 (IL-2, IFN-γ) and T2 (IL-4, IL-10) cytokine secretion

3.13.3 Donor/recipient chimerism studies:

a) Baseline determination in donor and patient (prior to start of induction chemotherapy with EPOCH-F/R).

b) After transplantation in patient:

- Peripheral blood lymphoid, myeloid, and total chimerism on day +14. Requires 10 ml of whole blood in EDTA.
- Peripheral blood and bone marrow total chimerism on days +28, +100, and 12 months. Lymphoid & myeloid subset chimerism will also be measured at these timepoints if donor chimerism was < 95% on the previous study.
- Chimerism may be measured at other timepoints if clinically indicated (e.g., to determine effect of manipulating immune suppression in order to increase donor chimerism).

4.0 Supportive Care

4.1 Infection Prophylaxis – See Appendix A for detailed description.

4.1.1 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. See Appendix A, Section 9.1.1 for details.

4.1.2 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 then continue through transplantation until day +100 or until immunosuppression is discontinued. See Appendix A, Section 9.1.2 for details.

4.1.3 Antibiotic therapy for neutropenic patients will be managed in accordance with CDC guidelines. Patients with neutropenia and fever that persists for longer than 5 days despite broad-spectrum antibiotics will receive empiric antifungal therapy with liposomal amphotericin B or with voriconazole, as described in Appendix A, Section 9.1.3.

4.1.4 All patients will receive valacyclovir for prophylaxis against herpes simplex virus and varicella zoster virus infection/reactivation. This therapy will start on day 1 of pre-transplant induction chemotherapy, continuing through transplantation until day +100 or until immunosuppression is discontinued. See Appendix A, Section 9.1.4 for details.

4.1.5 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 antigenemia and clinical evaluation. Positive

antigenemia will be treated according to the schema in Appendix A, Section 9.1.5. Weekly monitoring will continue through day +100 (longer for patients at risk – see Appendix A).

4.1.6 Patients receiving immunosuppression for chronic GVHD will receive pencillin V for prophylaxis against bacterial infections. All patients will undergo vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae*, beginning 12 months after transplantation. See Appendix A, Section 9.1.6 for details.

4.2 Management of Engraftment Syndrome

4.2.1 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 [73]. Diagnostic criteria and a treatment schema for engraftment syndrome are included in Appendix B.

4.3 Treatment of Graft-Versus-Host Disease:

4.3.1 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 [74]. See Appendix C for details concerning the grading and management of acute GVHD.

4.3.2 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.

4.3.3 In general, patients with ≥ Grade II acute GVHD will be treated with high-dose, systemic corticosteroids.

4.3.4 Patients who fail to respond satisfactorily 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.

4.4 Menses Suppression and Contraception – Pre-menopausal women who have not undergone hysterectomy will be placed on medroxyprogesterone acetate 10 mg/day orally to suppress menses. This therapy will begin with the first cycle of induction therapy and continue until platelet recovery after transplantation. (> 50,000/mm^3 without transfusion). Female transplant recipients will be advised to use contraception for at least 1 year after transplantation and to have their male partners use condoms. Male transplant recipients will be advised to use contraception, preferably condoms, for 1 year after transplantation.

4.5 Blood Product Support – 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). All blood products with the exception of the stem cell product will be irradiated.

4.5.1 Leucocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused leukocytes and decrease the risk of CMV infection [75].

4.6 Nutritional Support – When mucositis or GVHD prevents adequate PO intake, parenteral hyperalimentation will be instituted and discontinued under the

direction of the dietary service. Oral intake will resume when clinically appropriate under the supervision of the dietary service of the Clinical Center.

4.7 Anti-emetics – Anti-emetic usage will follow Clinical Center Guidelines as well as recommendations from the Pharmacy service.

4.8 Hematopoietic Growth Factor Support – All patients will start G-CSF 10μg/kg/day on day 0 starting after the stem cell infusion. The dose will be given subcutaneously (SC). G-CSF will be continued until the absolute neutrophil count (ANC) is ≥ 5000/μl for three consecutive days and will be resumed if ANC drops below 1000/μl.

5.0 Data Collection and Evaluation

5.1 Data Collection - Data will be prospectively collected and entered into the Medical Oncology Clinical Research Unit database.

5.2 Response Criteria

5.2.1 Complete Response (CR):

a) Non-Hodgkin's lymphoma or Hodgkin's lymphoma: complete disappearance of all detectable signs and symptoms of lymphoma for a period of at least one month. 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). 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). The spleen (or any other organ), if considered to be enlarged due to involvement by lymphoma before therapy and on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. Any macroscopic nodules in any organs detectable on imaging techniques should no longer be present. 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 [40].

b) Chronic lymphocytic leukemia: complete resolution of all 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.

c) Chronic myelogenous leukemia: Hematologic CR – normalization of peripheral blood counts (WBC < 10K/μl, platelets < 450K/μl), no immature cells on peripheral smear (blasts, promyelocytes, metamyelocytes). Cytogenetic CR – hematologic CR, with cytogenetic studies negative for Philadelphia chromosome (Ph). Molecular CR – hematologic and cytogenetic CR, with PCR studies negative for *bcr/abl*.

d) Other leukemias, myeloproliferative disorders, or myelodysplastic syndrome: normalization of peripheral blood counts and bone marrow morphology, fewer than 5% blasts in bone marrow; absence of specific

molecular or cytogenetic markers of disease that were previously present. In the case of myeloproliferative disorders, there must be absence of bone marrow fibrosis with normal hematologic parameters.

e) Multiple myeloma: absence of urine and serum M-components by immunofixation and electopheresis; normalization of immunoglobulins is not required. Clonal plasma cells in bone marrow <1% by PCR or flow cytometry. Normal serum calcium. No new bone lesions; no enlargement of existing lesions. Vertebral collapse or other pathological fracture due to osteoporosis or existing lesion does not prevent categorization as CR.

5.2.2 Complete Response/unconfirmed (CRu):

a) 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). 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. 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. Indeterminate bone marrow, if previously involved with lymphoma, will also be considered CRu.

5.2.3 Partial Response (PR):

a) Non-Hodgkin's lymphoma or Hodgkin's lymphoma: a 50% or greater decrease in SPD of all measured lesions lasting for a period of at least one month. No individual lesion may increase in size, and no new lesions may appear [40].

b) Chronic lymphocytic leukemia: a 50% or greater decrease in SPD of measured lymph nodes, hepatomegaly, or splenomegaly lasting at least 2 months, plus one or more of neutrophils $\geq$ 1.5K/μl, platelets > 100K/μl, or hemoglobin > 11g/dl (or 50% improvement).

c) Chronic myelogenous leukemia: Hematologic PR – as for 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. Cytogenetic PR – hematologic CR, with 1-34% Ph-positive cells (major response). Cytogenetic minor response – hematologic CR, with 35-90% Ph-positive cells.

d) Multiple myeloma: reduction by $\geq$ 75% in serum myeloma protein production, with decrease in Bence-Jones proteinuria by $\geq$ 90%. Clonal marrow plasmacytosis $\leq$ 5%. No new lytic bone lesions.

5.2.4 Stable Disease (SD):

a) Non-Hodgkin's lymphoma or Hodgkin's lymphoma: tumor measurements not meeting the criteria for CR, CRu, PR, or PD.

b) Chronic lymphocytic leukemia: response parameters not meeting criteria for CR, PR, or PD.

5.2.5 Relapsed or Progressive Disease (PD):

a) Non-Hodgkin's lymphoma or Hodgkin's lymphoma: a 25% or greater increase in SPD of all measured lesions compared to the smallest previous measurements, or appearance of any new lesion(s).

b) Chronic lymphocytic leukemia: a 50% or greater increase in SPD of all measured lesions compared to the smallest previous measurements, or appearance of any new lesion(s); a $\geq$ 50% increase in circulating lymphocytes; Richter's syndrome.

c) Chronic myelogenous leukemia: Increase in the number of metaphases demonstrating Ph by cytogenetics or t(9:22) by FISH; return to PCR positivity for *bcr/abl* after previously becoming negative.

d) Other leukemias, myeloproliferative disorders, or myelodysplastic syndrome: bone marrow and peripheral blood morphological features consistent with relapse or progression, including rising blast count and re-emergence of specific molecular or cytogenetic markers.

e) Multiple myeloma (requires 2 of the following): an increase in serum M-protein to $\geq$ 50% above the lowest level or a rise $\geq$ 2 g/dl; an increase in urine light chain excretion to 50% above the lowest value (at least 250 mg/24 hours) or an increase $\geq$ 2 g/24 hours of light chain excretion; an 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.

5.2.6 Refractory Disease:

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

5.3 Toxicity Criteria

5.3.1 The NCI Common Toxicity Criteria (Version 2.0, available via NCI web site: *http://ctep.info.nih.gov/CTC3/ctc.htm*) will be utilized for evaluation of toxicity during induction chemotherapy with EPOCH-F/R.

a) For patients with leukemia or bone marrow infiltrative/myelophthisic processes, hematologic toxicities during induction chemotherapy with EPOCH-F/R will be graded according to the specified criteria in CTC Version 2.0.

b) After patients begin the transplant preparative regimen (transplant day –6), hematologic and other toxicities will be graded where applicable according to the criteria specified for bone marrow transplantation studies in CTC Version 2.0.

5.4 Statistical Section:

5.4.1 There are no gender or racial/ethnic restrictions on patient selection. This protocol is open to all genders and racial/ethnic groups.

5.4.2 Statistical Considerations

5.4.3 The primary objectives of this study are to conduct an allogeneic HSCT protocol to identify if a more intensive GVHD prophylaxis regimen can be used with good results, and to assess the toxicity of a new induction chemotherapy regimen, EPOCH-F/R. Secondary objectives are to study the efficacy of the new induction regimen, to evaluate the fractions of patients with complete and mixed chimerism, and to study CD4 lymphopenia as a surrogate marker for the degree of immunosuppression required for engraftment after transplantation, and to determine an approximate level of correlation between serum IL-7 and IL-15 levels and circulating lymphocyte subpopulations.

5.4.4 The GVHD prophylaxis will primarily be evaluated in an overall fashion (i.e, in all patients enrolled on this trial); for this analysis, patients will not be stratified prospectively by induction chemotherapy received (i.e., with or without rituximab, actual dose level administered). This analysis is appropriate because the induction regimen is not expected to influence the development of GVHD (Section 1.2.7); relevant clinical risk factors for acute GVHD include the transplant conditioning and GVHD prophylaxis regimens, which are identical for all patients undergoing allogeneic HSCT in this study. A post-hoc analysis will be performed to verify that no relationship is observed between rituximab administration with the induction regimen and the development of acute GVHD. Data from CC 99-C-0143 and elsewhere suggest that 25 to 45% of patients like those enrolling in this trial will ordinarily develop grade III or IV GVHD with cyclosporine monotherapy for GVHD prophylaxis. We would like to determine if, with the GVHD prophylaxis regimen to be evaluated in this trial, we can obtain at least a slightly improved GVHD prophylaxis result in a limited number of subjects. To do so, the protocol will follow a standard Simon two-stage optimal design, with alpha=0.10, beta=0.10, p_0=0.55 (aiming to rule out 55% without grade III or IV GVHD) and p_1=0.80 (targeting 80% without grade III or IV GVHD). Stage I will initially enroll 8 patients; if no more than 4 of the first 8 patients enrolled in the study avoid grade III or IV acute GVHD, then the study will be halted. (In practice, a small number of additional patients may be accrued before the proportion of the first 8 patients experiencing grade III or IV acute GVHD can be determined.) If 5 or more of the first 8 patients evaluated (62.5% or more) avoid grade III or IV GVHD, then the study will continue accrual to a total of 26 patients. If 5 to 17 of 26 patients in the study avoid grade III or IV acute GVHD, this will be considered insufficient to conclude that the GVHD prophylaxis regimen is potentially better than that used in CC 99-C-0143. It should be noted, however, that it is unlikely that fewer than 14/26 patients will have acceptable GVHD, given that at least 5/8 were acceptable from the first accrual stage. Should 14-17 of 26 have less than grade III or IV GVHD, then we will use other information obtained from this trial and others to evaluate its potential utility. However, if 18 or more of 26 patients avoid grades III-IV acute

GVHD (69% or more), we will conclude that the GVHD prophylaxis regimen is potentially superior to that used in 99-C-0143.

An interim analysis of results through April 2004 suggested that the combination GVHD prophylaxis regimen being tested in this trial may demonstrate superiority over that used in 99-C-0143. As of this analysis, 12 of 17 patients evaluable through 100 days post HSCT had avoided grade III or IV acute GVHD (71%). Based upon these results, the accrual ceiling was increased by up to 6 additional patients in May 2004. Currently, 16 of 21 patients evaluable through 100 days post HSCT have avoided grade III or IV acute GVHD (76%). In view of these successful outcomes, the accrual ceiling will be increased so that up to 8 more patients may be enrolled to undergo allogeneic HSCT on CC 03-C-0077. The increased accrual target will permit a more precise estimate of the incidence of grade III or IV acute GVHD and will enable us to collect additional data on the more frequent occurrence of mixed donor-recipient chimerism post HSCT that we have observed with this prophylaxis regimen. In addition, the higher accrual capacity will support the continued development of our research program by extending this protocol's availability for eligible patients while our next allogeneic HSCT protocol is completing the process of regulatory approval.

5.4.5 The induction chemotherapy regimen will differ for patients with CD20 positive and CD20 negative malignancies, as described in Section 3.3. The main purpose of this study is to determine the effectiveness of the GVHD prophylaxis regimen; with respect to the induction regimen, this is a pilot study only. Our study population is expected to include patients with a broad range of characteristics, making it difficult to draw reliable conclusions by comparing efficacy and toxicity with the results seen in CC 99-C-0143. For these reasons, our analyses of efficacy and toxicity will be exploratory and will serve as the basis for designing subsequent studies. Subject to those provisos, we will determine the overall response rate of the induction regimen, with confidence intervals, as our principal evaluation of the EPOCH-F/R regimen's efficacy. This response rate will be compared informally with that observed with the EPOCH-F regimen in CC 99-C-0143. In addition, we will determine the response rate according to CD20 status and rituximab therapy, recognizing that the study design will not have sufficient statistical power to allow formal comparison of response between these subgroups. Similarly, our analysis of toxicity will be predominantly descriptive, as varying patient risk factors will render more formal analysis difficult to interpret. We will determine the worst overall toxicity per patient as well as the individual toxicities of each type per cycle and report these and other descriptive results; this analysis will be conducted primary in an overall fashion. Although we do not predict that rituximab therapy will contribute significantly to toxicity, our analysis will be stratified according to the use of rituximab to explore any possible relationship.

5.4.6 For patients with disease progression after transplantation who receive additional conventional therapies (chemotherapeutics, cytokines, or monoclonal antibodies) and/or donor lymphocyte infusion (DLI), we will determine the overall response rate to such therapies, with confidence intervals. Further evaluation of response rates may be performed according to the type of salvage therapy received (conventional agents, DLI, or both) and according to response to induction chemotherapy, if the number of patients receiving salvage therapy is sufficient to permit meaningful analysis.

5.4.7 Based on results in CC 99-C-0143, we anticipate that a high percentage of patients will engraft with early conversion to full donor chimerism. Thus, we will compare the results obtained on this trial to those of the previous study and other relevant trials. The following stopping rule will be employed with regard to graft rejection or failure to engraft:

	Engraftment Failure or Graft Rejection Cumulative # events observed	Action
Patients 1 through 10	> 1	Suspend accrual
Patients 11 through 20	> 2	Suspend accrual
Patients 21 through 26	> 3	Suspend accrual

The fractions of patients with mixed chimerism and complete chimerism will be determined and reported with a 95% confidence interval. These results will also be stratified according to the use of rituximab with induction chemotherapy, but will primarily be evaluated in an overall fashion. If graft rejection or mixed chimerism is seen, then CD4, CD8 and absolute lymphocyte counts will be compared between patients who engrafted or not using a Wilcoxon rank sum test, and we will consider using logistic regression to try to identify, in a limited manner given the size of this study, which factors are best associated with successful engraftment, possibly adjusting for other clinical factors as appropriate. In addition, Spearman correlation will be used to address the association between serum IL-7 levels, IL-15 levels, and lymphocyte subpopulations.

5.4.8 It is expected that accrual to this protocol can be completed in 2 years if approximately 1 patient per month is enrolled.

5.4.9 Kaplan-Meier estimates of overall and progression free survival will be determined, but will be interpreted with appropriate cautions given the small sample size of the study.

5.5 Data and Safety Monitoring Plan

5.5.1 In accordance with NCI IRB policies, this pilot study will not require independent monitors or review by a Data Safety and Monitoring Board.

5.5.2 The PI and LAI will provide continuous, close monitoring, with prompt reporting of serious adverse events to the IRB (Section 7.1). On a weekly basis, the PI, LAI, Protocol Research Nurse, and Transplant Coordinator will review clinical and laboratory data from each of the transplant recipients. A complete summary of patient outcomes will be provided

annually to the IRB. The Protocol Support Office of the Medical Oncology Clinical Research Unit, CCR, NCI will assist with annual internal auditing of subject case records. After accrual of 3 subjects, the Protocol Support Office will also perform a protocol activation audit to review adherence to informed consent procedures, eligibility documentation, and initial treatment.

5.5.3 The expected heterogeneity of study participants with respect to malignancy and risk factors for toxic events renders these parameters impractical to evaluate in a statistical manner; accordingly, formal stopping rules will not incorporate toxic events but will instead be based upon the rate and severity of acute GVHD, as described in Section 5.4.

6.0 Human Subject Protections

6.1 Rationale for Subject Selection: Patients with refractory lymphoid malignancies, leukemia, myelodysplasia, and myeloproliferative disorders will be the subjects for this study. Conventional chemotherapy is seldom curative for patients with the disorders selected for inclusion in this study (section 2.1.1). Allogeneic HSCT represents a potentially curative treatment for these diseases, which occur most frequently in individuals greater than 40 years of age. However, the considerable risk of early treatment-related mortality related to conventional preparative regimens increases with patient age. The use of a reduced-intensity preparative regimen in patients with these conditions will potentially reduce treatment-related mortality without a reduction in efficacy.

6.2 Risks/Benefits Analysis: There is potential benefit to the transplant recipient, as demonstrated by the published literature documenting long-term disease-free survival for patients with refractory lymphoid malignancies, leukemias, myeloproliferative disorders, and myelodysplasia following allogeneic HSCT. The use of a reduced-intensity preparative regimen may permit patients to undergo allogeneic HSCT with fewer toxicities than if a conventional preparative regimen were used; this potential benefit increases with the age of the transplant recipient (see section 6.1). However, we do not anticipate any direct benefit to the other subject covered by this protocol, i.e., the stem cell transplant donor.

6.2.1 The acute risks to the stem cell donor include those associated with venipuncture and apheresis.

6.2.2 Potential risks to the transplant recipient have also been considered and are felt to be potentially less than would otherwise occur in conventional allogeneic HSCT. The risk of both acute and chronic GVHD will be present, as discussed previously.

6.2.3 Children under the age of 12 years will be excluded as potential donors, given the uncertain risks in this young population and the low likelihood that such potential donors will be encountered.

6.3 Consent and Assent Process and Documentation:

6.3.1 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.

6.3.2 Consent forms: 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 the donor.

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

7.0 Data Reporting

7.1 Adverse Reactions - Reports of adverse reactions will be made using the Division of Cancer Treatment Common Toxicity Criteria (Version 2.0) for reference according to the guidelines published by the DCTD, NCI. A copy of the CTC version 2.0 can be obtained from the CTEP home page (http://ctep.info.nih.gov). These guidelines are summarized as follows:

7.1.1 Report by telephone to NCI IRB within 24 hours (301-230-2330):

a) All life-threatening events (i.e., grade 4, except for myelosuppression), which may be due to administration of the study drug(s).
b) All failures to engraft, or occurrences of graft rejection.
c) All fatal events (grade 5).

7.1.2 A written report should follow within 10 working days to:

NCI Institutional Review Board
Center for Cancer Research
National Cancer Institute
Building 82, Room 117
Bethesda, MD 20892

Serious adverse events that occur with commercially available agents will also be reported directly to the FDA through the MedWatch system (http://www.fda.gov/medwatch/).

7.2 Record Keeping

7.2.1 All patients (donor and recipient) must have signed an Informed Consent, and on-study confirmation of eligibility form will be filled out before entering on the study.

8.0 Pharmaceutical Information

8.1 CYCLOPHOSPHAMIDE (CTX, Cytoxan, NSC-26271)

8.1.1 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.

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

8.1.3 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.

8.1.4 Route of Administration - The cyclophosphamide used in this regimen will be mixed in 100 ml normal saline and given as an IVPB over 30 minutes in the induction regimen (i.e. EPOCH-F/R; Section 3.3.2). In the preparative regimen (Section 3.4.4), it is given over two hours.

8.1.5 Toxicities:

a) Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].

b) Water retention – cyclophosphamide 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.

c) Cardiomyopathy - cyclophosphamide 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.

d) 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 and the administration of Mesna. Diuresis is maintained for 24 hrs after completion of last dose by parenteral infusions of normal saline with potassium chloride, as specified in section 3.4.4. 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.

e) Sterility

f) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

8.2 MESNA (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

8.2.1 Supply – Mesna will be will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a 100mg/ml solution.

8.2.2 Preparation – Dilute up to 20 mg Mesna/ml fluid in D5W or normal saline. Mesna should be started one hr prior to the cyclophosphamide in the

preparative regimen (Section 3.6). Bag #1 of the Mesna will be 150 mg/m^2 IV in 250 ml, given as a 3 hour infusion (thus stopping when cyclophosphamide ends). Then immediately afterward, Mesna will be given at 1200 mg/m^2 in 500 ml by continuous IV infusion over 24 hour infusion for four doses (days -6, -5, -4, and -3).

8.2.3 Storage and Stability – Intact ampules are stored at room temperature. Diluted solutions (1 to 20 mg/dl) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% normal saline, or 24 hours in normal saline.

8.2.4 Administration - To be administered intravenously as continuous infusion.

8.2.5 Toxicities - Nausea, vomiting, diarrhea. A very rare complication is cough, dyspnea and fever with rapid progression to diffuse pulmonary fibrosis and death.

8.3 FILGRASTIM (G-CSF, Neupogen®)

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

8.3.2 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 with saline 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.

8.3.3 Storage and Stability – Filgrastim for injection should be stored in the refrigerator at 2^o to 8^{o}C (36^o to 46^{o}F). Avoid shaking.

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

8.3.5 Toxicities – Medullary bone or skeletal pain is the most commonly reported toxicity. In addition, reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase are common laboratory abnormalities. 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.

8.4 CYCLOSPORINE (Gengraf, Sandimmune, Neoral)

8.4.1 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.

8.4.2 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 8.4.2). Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.

8.4.3 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.

8.4.4 Administration – Cyclosporine may be given intravenously over 2 hours or orally.

8.4.5 Toxicities – 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. 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. Hypertension occurs in up to 60% of patients. Hypomagnesemia can be associated with neurologic symptoms, including seizures, cerebellar ataxia and depression. Dose-related hepatotoxicity, manifested by elevation of serum transaminases and bilirubin, has been reported.

8.5 FLUDARABINE (Fludara, Berlex Laboratories)

8.5.1 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.

8.5.2 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.

8.5.3 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.

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

8.5.5 Toxicities – Fludarabine toxicities include myelosuppression (dose limiting toxicity), 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.

8.6 ETOPOSIDE, DOXORUBICIN, and VINCRISTINE (in EPOCH)

8.6.1 Supply – Etoposide, doxorubicin, and vincristine will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

8.6.2 Preparation and Administration - 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
≤150 mg	528 mL	25 mL	528 mL	22 mL/hour

≥151 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.

8.6.3 Storage and Stability - 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 [76].

8.6.4 Toxicities–

a) Etoposide: nausea, vomiting, stomatitis, diarrhea, neutropenia, thrombocytopenia, and alopecia. Secondary AML has been associated with etoposide. Bradycardia and hypotension are sometimes observed with etoposide administration.

b) Doxorubicin: Cardiotoxicity is particularly noted after cumulative doses of greater than 550 mg/m^2. Other toxicities include myelosuppression, nausea, vomiting, stomatitis, diarrhea, and alopecia. Skin infiltration of doxorubicin causes tissue necrosis.

c) Vincristine causes neurological toxicities such as paresthesias, jaw pain, ataxia, foot-drop, cranial nerve palsies, paralytic ileus, constipation, abdominal pain, and loss of deep tendon reflexes. It is also a vesicant, and occasionally causes alopecia and myelosuppression.

8.7 PREDNISONE

8.7.1 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.

8.7.2 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.

8.7.3 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.

8.7.4 Toxicities - Prednisone frequently causes gastritis, immunosuppression, muscle wasting, fluid retention, and hyperglycemia.

8.8 METHOTREXATE (Methotrexate sodium, MTX, NSC-740)

8.8.1 Supply – Methotrexate will be obtained commercially and is supplied as a 25 mg/ml preservative-free isotonic solution for injection.

8.8.2 Preparation – The desired dose will be diluted in 5% dextrose in water or 0.9% sodium chloride.

8.8.3 Storage and Stability – Methotrexate should be stored at room temperature and protected from light. Once the prepared dose is diluted for administration, the solution is stable for 24 hours refrigerated or at room temperature when protected from light.

8.8.4 Administration – Methotrexate will be given as IV infusion over 15 minutes.

8.8.5 Toxicities - The toxicity associated with methotrexate primarily involves the gastrointestinal tract. Severe mucositis can occur, particularly in patients who have received an intensive preparative regimen. Other gastrointestinal symptoms include nausea, vomiting and diarrhea. Transient elevations in serum transaminases have been seen. The myelosuppression associated with methotrexate often results in delayed engraftment. Folinic acid can effectively circumvent the enzymatic block produced by methotrexate.

8.9 RITUXIMAB (Rituxan)

8.9.1 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.

8.9.2 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.

8.9.3 Storage and Stability – Rituximab vials should be stored at 2–8°C (36–46°F) and should be protected from direct sunlight.

8.9.4 Administration – 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.

8.9.5 Toxicities – 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.

a) 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.

b) 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.

c) 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.

d) 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 saline, 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).

8.10 DIPHENHYDRAMINE

8.10.1 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.

8.10.2 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.

8.10.3 Storage and Stability – Store commercially available injectable product at controlled room temperature.

8.10.4 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).

8.10.5 Toxicities – 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.

8.11 ACETAMINOPHEN

8.11.1 Supply – Commercially available as 325 mg or 500 mg tablets for oral administration from multiple manufacturers.

8.11.2 Storage – Store at controlled room temperature.

8.11.3 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.

8.11.4 Toxicities – No toxicities are anticipated to result from single doses of acetaminophen administered as premedication for rituximab infusions.

8.12 VALACYCLOVIR (Valtrex®)

8.12.1 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.)

8.12.2 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.

8.12.3 Storage and Stability – Oral tablets should be stored at 15^{o} to 25^{o}C (59^{o} to 77^{o}F).

8.12.4 Administration – Oral.

8.12.5 Toxicities – Nausea and/or vomiting, headache, dizziness, abdominal pain, dysmenorrhea, arthralgia, acute hypersensitivity reactions, elevations in liver enzyme laboratory values (e.g. AST). Renal failure and CNS symptoms have been reported in patients with renal impairment who received valacyclovir 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).

8.13 FLUCONAZOLE (Diflucan®)

8.13.1 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).

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

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

8.13.4 Administration – Oral and parenteral. Parenteral doses should be administered by an intravenous infusion at a maximum rate of 200mg/hr.

8.13.5 Toxicities – Nausea, vomiting, headache, skin rash, abdominal pain, diarrhea have been reported at an incidence of 1% or greater in clinical trials. 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.

8.13.6 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.

8.14 TRIMETHOPRIM/SULFAMETHOXAZOLE (TMP/SMX, Cotrimoxazole, Bactrim, Septra)

8.14.1 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.

8.14.2 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. Normal saline (0.9% sodium chloride) may be substituted as a diluent but the resulting solutions have reduced stability. Consult with pharmacy for questions regarding diluent, volume, and expiration.

8.14.3 Storage and Stability – Oral tablets and oral suspension should be stored at 15° to 30°C (59° to 86°F) in a dry place and protected from light. TMP/SMX for injection should be stored at room temperature between 15°

to 30°C (59° to 86°F) 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.

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

8.14.5 Toxicities – The most common adverse effects from TMP/SMX are gastrointestinal disturbances (nausea, vomiting, anorexia) and allergic skin reactions (such as rash and urticaria). 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. For TMP/SMX injection, local reaction, pain and slight irritationon IV administration are infrequent. Thrombophlebitis has rarely been observed.

8.15 MEDROXYPROGESTERONE ACETATE (Provera)

8.15.1 Supply – Commercially available as 2.5mg, 5mg, and 20mg tablets.

8.15.2 Storage – Tablets should be stored at controlled room temperature 20° to 25°C (68° to 77°F).

8.15.3 Administration – Oral.

8.15.4 Toxicities – 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.

8.16 IMMUNE GLOBULIN INTRAVENOUS (IGIV)

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

8.16.2 Preparation – Refer to the manufacturer's package labeling for guidelines in preparation.

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

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

8.16.5 Toxicities:

a) 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. 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.

b) 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.

c) 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.

d) An aseptic meningitis syndrome has been reported to occur infrequently in association with IGIV treatment.

e) 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.

9.0 Appendices

9.1 Appendix A: Prophylaxis and Treatment of Infectious Complications in Allogeneic HSCT Recipients

Note: The practice guidelines included in this Appendix are based upon the best available clinical evidence and may change as additional data become available.

9.1.1 *Pneumocystis carinii* pneumonia

f) 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.

g) Upon admission for the transplant conditioning regimen (day –7), all patients will receive TMP/SMX 160/800, one tablet PO BID, continuing daily through the evening of day –2.

h) Prophylaxis is resumed 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. Note: TMP/SMX should not be resumed before the last day of methotrexate administration (day +11 – see Section 3.5.3). 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 allergic to sulfa, see discussion in "Rationale", below.

Rationale:

- PCP is common (incidence 21 cases per 100 patients/year in the absence of prophylaxis), potentially lethal and preventable. It is not known when it is safe to discontinue prophylaxis, but CDC guidelines recommend prophylaxis for 6 months after transplant unless there is persistent immunosuppression (i.e., steroids). In a case series reported in 1996, late PCP happened only in patients receiving steroids for GVHD [77]7].
- The CDC recommends TMP/SMX 160/800 daily OR 80/400 daily OR 160/800 three days/week. The "weekend" regimen has not been shown to be equivalent in RCT, but has been used widely in BMT and solid organ transplant.
- Chronic GVHD in the absence of active immunosuppressive treatment probably does not require PCP prophylaxis. A convenient alternative in this setting is TMP/SMX 80/400 daily, as this provides prophylaxis for PCP and bacterial infections.
- The alternative regimens for PCP prophylaxis are less effective than TMP/SMX. Pentamidine inhalation 300 mg/4 weeks is felt to be the least effective regimen [78]8]. There is ample documentation in the HIV literature that pentamidine 300 mg q 2 weeks is more effective than monthly, so this may be considered an option in selected cases [79-81]1]. Dapsone is the recommended alternative, but its use is often impractical due to occasional cross-reactivity with TMP/SMX. Also, dapsone has known hepatotoxicity, so it may be difficult to use in patients with already abnormal liver enzymes. Atovaquone has been used successfully in liver transplantation [82]2], HIV [83, 84]4] and autologous BMT [85]5].

FUNGAL PATHOGENS

9.1.2 Yeast Infections

a) 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 then be restarted on day 6 of each cycle.
b) 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.

Rationale:
The CDC Guidelines recommend fluconazole until day 30 (or 7 days of ANC > 1000/μl) only. However, the long-term follow-up [86] of the Seattle study that showed decreased fungal infections and improved survival [87] suggests that fluconazole for 75 days protects from gut GVHD, disseminated candidal infections and candidiasis-related death. For this reason we prefer to continue fluconazole administration until day 100 or the end of immunosuppressive treatment, whichever is longer.

9.1.3 Mould Infections (including *Aspergillus*)

a) During Neutropenia: Neutropenic patients who are febrile for more than 5 days despite broad-spectrum antibiotics and have no clinical evidence of invasive fungal disease will receive liposomal amphotericin B (Ambisome) 5 mg/kg/day or voriconazole 6 mg/kg q12h for two doses and then 4 mg/kg q12h. This therapy will continue until the patient attains an ANC > 500/μl AND is afebrile for 24 hours. Fluconazole prophylaxis will be discontinued during this therapy.

Rationale:
The empirical use of antifungal therapy during neutropenia is controversial. Less than 5% of patients with persistent fever during neutropenia have a documented fungal infection. Only one RCT has shown decreased fungal infection-related death with the empirical addition of amphotericin B in neutropenic fever, and this study actually failed to show an overall survival advantage [88]. The relevance of these results to patients with short neutropenia (median = 9 days) who are receiving fluconazole prophylaxis is questionable. The most recent trial of empirical antifungal therapy in febrile neutropenic patients showed 19 fungal infections in 837 patients at the time of initiating antifungal therapy and another 29 documented "breakthrough" invasive fungal infections during follow-up. In the high-risk subgroup (relapsed leukemia and/or allogeneic bone marrow transplant), the prevalence of breakthrough fungal infections was higher (15/289) [89]. Our recommendations are based on the following reasoning: if persistent fever during neutropenia is a manifestation of an occult fungal infection in our patients (severely immunosuppressed, with short neutropenia and long-term use of fluconazole), then the most likely pathogens (fluconazole-resistant Candida and Aspergillus) are probably better treated with high-dose liposomal amphotericin B (at least 5 mg/kg). As all the allogeneic HSCT patients receive cyclosporine after transplantation, liposomal amphotericin B is the lipid formulation of choice. Voriconazole is a valid alternative, and in the mentioned study was associated with significantly fewer breakthrough fungal infections than Ambisome [89].

VIRAL PATHOGENS

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

a) 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 chemotherapy (EPOCH-F/R) and continue throughout transplantation. Valacylovir 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.

Rationale:

- HSV is an important cause of morbidity after high-dose chemotherapy. Several effective regimens are available. Every patient receives prophylaxis regardless of HSV serology because of the possibility of primary infection after the test, and the fact that valacyclovir may also prevent VZV reactivation. There is no compelling reason to try to prevent HSV beyond the period of mucositis, but our patients will remain on valacyclovir for a minimum of 6 months.
- VZV reactivates in a significant proportion of allogeneic HSCT recipients once antiviral agents are stopped (30-60% in the first year). The morbidity associated with VZV reactivation is high. Hence, prophylaxis with antiviral agents is accepted practice ("...this therapy could be considered for use among HSCT recipients with severe, long-term immunodeficiency"[90]). Low-dose acyclovir (400 mg/d) administered until the end of immunosuppressive therapy has been reported to significantly decrease the incidence of reactivation [91]. It is not known which antiviral and what dose are best to prevent VZV, or for how long to administer it. The universal experience seems to be that reactivation is unusual for as long as patients are on any prophylactic anti-herpesvirus agent [92, 93]. We prefer valacyclovir because of the convenience of once daily dosing, although it is not FDA-approved for this indication.

9.1.5 Cytomegalovirus (CMV)

a) Preemptive Therapy Before Day +100: Patients with positive pre-transplant serologies for CMV, or whose donors have positive serologies, will undergo weekly monitoring of CMV antigenemia. Antigenemia monitoring will begin at the time of engraftment after transplantation and continue through day +100. If a patient has an ANC < 1000/μl, CMV monitoring will be performed via polymerase chain reaction (PCR) instead of antigenemia. If antigenemia becomes positive (expressed as the number of positive cells per 400,000 white blood cells) before day +100, then patients will be treated with oral valganciclovir, IV ganciclovir, or IV foscarnet according to the following schema:

# Of (+) cells	Rx: VALGANCICLOVIR	Rx: IV GANCICLOVIR	Monitoring	Reinduction
Any	**Valganciclovir induction:** 900 mg PO q12h for 7 days**,** then **maintenance:** 900 mg/24h until antigenemia is negative x 2 weeks*	**Ganciclovir induction:** 5 mg/kg IV q12h for 7 days**,** then **maintenance:** 5 mg/kg/24h 5 days/week until antigenemia is negative x 2 weeks*	Weekly antigenemia	1. If antigenemia becomes positive after being negative 2. For doubling of antigenemia

*I.e., on two consecutive weekly antigenemia tests. This may include tests performed during the two-week period of induction therapy.

NOTES:

- Valganciclovir and ganciclovir may cause bone marrow suppression. If ANC < 1000: Start filgrastim (G-CSF) 5-10 µg/kg/d
- Weekly IVIG infusion has no proven benefit in the setting of CMV reactivation without established infection. Therefore, its use will be limited to patients with CMV pneumonitis (or other target organ infection, at the investigators' discretion).

Indications for IV ganciclovir:

- GVHD with diarrhea or clinical suspicion of malabsorption

Indications for foscarnet:

- ANC < 500/µl despite G-CSF
- Platelets < 20,000/mm^3
- Resistance to ganciclovir: rising antigenemia after 3 weeks with no response to ganciclovir reinduction.

Dose Adjustments for Renal Insufficiency

Valganciclovir		
Calculated CrCl	**Induction**	**Maintenance**
>60	900 mg q12h	900 mg q24h
40-59	450 mg q 12h	450 mg q 24h
25-39	450 mg q 24h	450 mg q 48h
10-24	450 mg q 48h	450 mg 2x/week

Ganciclovir		
Calculated CrCl	**Induction**	**Maintenance**
>70	5 mg/kg q12h	5 mg/kg q24h
>50-69	2.5 mg/kg q12h	2.5 mg/kg q24h
25-49	2.5 mg/kg/24h	1.25 mg/kg/24h
10-24	1.25 mg/kg/24h	0.625 mg/kg/24h
<10	1.25 mg/kg 3x/wk	0.625 mg/kg 3x/wk

b) Preemptive Therapy After Day +100: Patients who remain at risk for CMV reactivation after day +100 will continue to have antigenemia (or PCR) monitoring performed weekly. These patients include:

- Patients with chronic GVHD and systemic steroid therapy
- Patients undergoing transplantation from unrelated donors
- Patients receiving T cell-depleted allogeneic HSCT

Note that pre-emptive therapy after day +100 differs in both threshold and duration from that administered before day +100. The treatment schema follows:

# Of (+) cells	Rx: VALGANCICLOVIR	Rx: IV GANCICLOVIR	Monitoring	Reinduction
1 cell	**None**	**None**	Repeat antigenemia in 3 days	
> 1 cell	**Valganciclovir induction:** 900 mg PO q12h for 7 days**,** then **maintenance:** 900 mg/24h until antigenemia is negative x 2 weeks*	**Ganciclovir induction:** 5 mg/kg IV q12h for 7 days**,** then **maintenance:** 5 mg/kg/24h 5 days/week until antigenemia is negative x 2 weeks*	Weekly antigenemia	1. If antigenemia becomes positive after being negative 2. For doubling of antigenemia

*I.e., on two consecutive weekly antigenemia tests. This may include tests performed during the two-week period of induction therapy.

Rationale:
Valganciclovir is has proven similar efficacy to IV ganciclovir in a randomized controlled trial of therapy for retinitis in AIDS [94]. Both drugs have NOT been shown to be equivalent in the setting of allogeneic HSCT, and the preferred treatment is IV ganciclovir as per the CDC guidelines [95]. In particular, when there is a strong concern with drug absorption, ganciclovir is the treatment of choice. Others and we have used valganciclovir successfully during the national shortage of ganciclovir, but there is still no strong evidence to support its use for this indication.

9.1.6 Bacterial Pathogens

a) Prophylaxis During Chronic GVHD: All patients receiving treatment for chronic GVHD will receive penicillin V 500 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.

Rationale:
There is an increased risk of infection caused by encapsulated organisms, particularly *Streptococcus pneumoniae* in patients with chronic GVHD [96]. Functional hyposplenism has been considered, and some investigators have attempted to monitor it by ultrasound [97]. Current recommendations support the use of antibiotic prophylaxis with penicillin to prevent these infections [90]. TMP/SMX could be substituted, but only when administered daily.

b) Vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae*: All patients will receive *Streptococcus pneumoniae* 23-valent polysaccharide vaccine (Pneumovax) at 12 and 24 months, and Hib vaccine at 12, 14 and 24 months after transplant. An alternative

vaccine against *Streptococcus pneumoniae* may be adopted when it becomes available.

Rationale:
- The Hib vaccine has been shown to induce neutralizing antibodies effectively [98].
- The pneumococcal vaccine may be effective and is safe.

9.2 Appendix B: Management of Engraftment Syndrome (ES)

Clinical Definition of Engraftment Syndrome

A constellation of clinical symptoms and signs has been observed during neutrophil recovery following HSCT, most commonly termed "engraftment syndrome" (ES), but interchangeably termed "capillary leak syndrome" and "autoaggression syndrome". Engraftment syndromes have been observed after both autologous and allogeneic HSCT; in the latter setting, the clinical sequelae of neutrophil recovery were historically attributed to an early, sometimes "hyperacute" graft-versus-host reaction. Our current understanding of ES holds the overproduction of pro-inflammatory cytokines at the time of neutrophil recovery to be the initiating event. The study of ES has been somewhat hindered by the lack of uniform definition for this clinical entity; therefore, the following criteria have been proposed [73]:

Major criteria:	• Fever $\geq$ 38.3° with no identifiable infectious etiology • Erythrodermatous rash involving more than 25% of body surface area and not attributable to a medication • Noncardiogenic pulmonary edema, manifested by diffuse pulmonary infiltrates and hypoxia
Minor criteria	• Hepatic dysfunction with either 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

A diagnosis of ES is established by the presence of all three major criteria, or two major criteria and one or more minor criteria; the clinical signs and symptoms should appear within 96 hours of neutrophil recovery, according to the above proposed definition.

Treatment of Engraftment Syndrome:

The mainstay of therapy for ES is high-dose corticosteroids. This approach is derived from the literature on diffuse alveolar hemorrhage [99] in the setting of bone marrow transplantation – a complication that many investigators now believe to be part of the spectrum of ES. Our group has adopted the following treatment schema for patients diagnosed with ES, with satisfactory results:

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

In the event that symptoms or clinical signs of ES recur during the steroid taper, patients should be retreated with methylprednisolone at a minimum dose of 60 mg IV QD. *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.*

9.3 Appendix C: Grading and Management of Acute Graft-Versus-Host Disease

Clinical Staging of Acute GVHD [74]

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 of Acute GVHD [74]

	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 of Acute GVHD

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:
    a) 50 mg/m^2 per dose IV BID for 2 days.
    b) 37.5 mg/m^2 per dose IV BID for 2 days.
    c) 25 mg/m^2 per dose IV BID for 2 days.
    d) 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.
    e) 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.
    f) If GVHD worsens during taper, steroids should be increased to previous dose.
    g) During steroid taper, maintain cyclosporine at therapeutic levels (Section 3.5.1).
4) If no response is observed within 7 days of MP treatment:
    a) Increase Methylprednisolone to 500 mg/m^2 per dose IV, BID for 2 days.

b) 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 the treatment. The following are criteria to determine definitions of response to GVHD treatment:
    a) Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD.
    b) Partial Response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of adequate performance status (Karnofsky Score $\geq$ 70%).
    c) 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%).
    d) Progressive disease: Further progression of signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

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