Abbreviated Title: RIST using Unrelated Donors
NCI Protocol Number: 07-C-0195 X
Version Date: September 14, 2018
NCT Number: NCT00520130

PROTOCOL TITLE: Phase II Trial of Targeted Immune-Depleting Chemotherapy and Reduced-Intensity Allogeneic Hematopoietic Stem Cell Transplantation Using 8/8 and 7/8 HLA-matched Unrelated Donors and Utilizing Two Graft-versus-Host Disease Prophylaxis Regimens for the Treatment of Leukemias, Lymphomas, and Pre-malignant Blood Disorders

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Investigator Roles:

A. Obtain information by intervening or interacting with living individuals for research purposes
B. Obtaining identifiable private information about living individuals
C. Obtaining the voluntary informed consent of individuals to be subjects
D. Makes decisions about subject eligibility
E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes
F. Studying, interpreting, or analyzing coded (linked) data or specimens for research purposes
G. Some/all research activities performed outside NIH

Commercial Agents: Cyclophosphamide, Mesna, Fludarabine, Etoposide, Vincristine, Doxorubicin, Rituximab, Cytarabine, Methotrexate, Sirolimus, Tacrolimus, Alemtuzumab

PRÉCIS

Background:

- The major limitations to the broader applicability of allogeneic hematopoietic stem cell transplantation (HSCT) for the treatment of malignancies are lack of suitable donors and therapy-related toxicities which include delayed and incomplete immune reconstitution and graft-versus-host disease (GVHD). Based on the theory that the rapid establishment of donor chimerism was essential for an optimal "graft-versus-tumor" effect, we have employed a strategy of targeted immune-depleting chemotherapy prior to reduced-intensity allogeneic HSCT. It is our intent to investigate this approach in the setting of HLA-matched unrelated donors in a pilot manner.
- A clearly superior GVHD prophylaxis regimen has not been established in the unrelated donor transplant setting. The best results that have been reported are with the combination of alemtuzumab plus cyclosporine [AC] and the combination of tacrolimus, methotrexate, and sirolimus [TMS]. These two regimens work by mechanisms which are biologically distinct and potentially have markedly different effects upon immune reconstitution that have not been well studied. In addition neither of these regimens has been assessed for their effects on chronic GVHD using the NIH Consensus Conference Criteria. It is our intent to study the effects that these two regimens have on immune reconstitution and chronic GVHD in the setting sequential targeted immune-depleting chemotherapy and reduced-intensity allogeneic HSCT from HLA-matched unrelated donors.

Objectives:

- Primary objectives:

1) To assess the effects of two biologically distinct GVHD prophylaxis regimens, TMS and AC, on immune reconstitution in patients receiving targeted-immune depletion and reduced-intensity allogeneic HSCT from HLA-matched unrelated donors. As part of a comprehensive assessment of immune reconstitution, the primary immunologic endpoint will be the determination of CD4+ T cell receptor Vβ repertoire by CDR3 spectratyping at 3 months, 6 months, and 12 months post-transplant. Samples will be collected on each of the patients enrolled in the study. However, due to the time and cost involved in the spectratyping analysis, a comprehensive assessment will initially be limited to the first 10 patients on each arm receiving 10 of 10 HLA-matched unrelated donors After completion of this assessment, it will be determined whether or not the analysis should be continued.
2) To assess overall safety of these two regimens in this setting, as determined by engraftment, acute GVHD, early and late treatment-related mortality, and overall survival.
3) To determine and monitor incidence, organ severity and overall severity of chronic GVHD prospectively using the newly developed NIH Consensus Conference diagnosis and staging criteria and preliminarily validate those tools for use in clinical practice and trials (Pavletic, Imanguli, and Cowen).

Eligibility:

- Adults (18 – 74 years) with advanced or high risk hematologic malignancies including AML, ALL, MDS, CLL, NHL, HL, CML, multiple myeloma, and MPD who lack a suitable HLA-matched sibling.
- An unrelated donor matched at a minimum of 7 of 8 alleles (HLA-A,-B,-C, and DRB1) by high-resolution typing, identified through the National Marrow Donor Program.
- Life expectancy of at least 3 months, ECOG $\leq$ 2 and relatively normal major organ functions.

Design:

- Patients will receive disease-specific induction chemotherapy (EPOCH-F/R or FLAG) prior to transplant for disease control and immune depletion. If disease is controlled ($\geq$ SD) and immune depletion objectives have been met, patients may forgo induction chemotherapy and move forward to the transplant conditioning regimen.
- All patients will receive an identical conditioning regimen consisting of cyclophosphamide 1200 mg/m^2/day IV for 4 days and fludarabine 30 mg/m^2/day for 4 days.
- Patients will be stratified according to degree of HLA-match and randomized at the time of enrollment to one of two GHVD prophylaxis regimens:
  - Arm A (TMS): Tacrolimus, starting day -3 before transplant, given initially at 0.02 mg/kg/day CIV and then an equivalent oral dose (when patient taking po) titrated for a goal level of 5-10 ng/ml; sirolimus given as an initial loading dose of 12 mg p.o. on day -3 pre-transplant, 4 mg starting day -2 pre-transplant titrated for levels 3-12 ng/ml; and methotrexate 5 mg/m^2 IV on days +1, +3, +6, and +11 post-transplant. Tacrolimus and sirolimus will be tapered at day +63, day +119 and day +180 post-transplant as tolerated.
  - Arm B (AC): Alemtuzumab at 20 mg/day IV over 8 h on days −8 to −4 pre-transplant and cyclosporine, starting day -1 before transplant, given initially at 2 mg/kg IV every 12 hours and then an equivalent oral dose (goal level 150-250 ng/ml) until day 100. Cyclosporine will be tapered by 10% per week between day 100 and six months).
- A maximum of 105 patients will be enrolled and randomly assigned to the two arms in order to yield 44 patients per arm (88 total patients) who are able to be evaluated for development of severe chronic GVHD. This represents an increase of 20 patients over the number permitted prior to this amendment: 25 patients (50 total) randomly assigned to each arm within the 8/8 match group and 13/arm (26 total) within the 7/8 match group (maximum study enrollment = 76 transplanted patients from among up to 85 patients registered).

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives:

1.1.1.1 To assess in a pilot manner the effects of two biologically distinct graft-versus-host disease (GVHD) prophylaxis regimens (tacrolimus/methotrexate/sirolimus [TMS] and alemtuzumab/cyclosporine [AC]) on immune reconstitution in patients receiving targeted-immune depletion and reduced-intensity allogeneic hematopoietic stem cell transplantation (HSCT) from HLA-matched unrelated donors. As part of a comprehensive assessment of immune reconstitution, the primary immunologic endpoint will be the determination of CD4+ T cell receptor Vβ repertoire by CDR3 spectratyping at 3 months and 12 months post-transplant (Friedman and Korngold). This data will be complemented by additional analyses on immune reconstitution as defined by the first five secondary objectives.

1.1.1.2 To assess in a pilot manner the overall safety, as determined by engraftment, severe (Grade III-IV) acute GVHD, early and late treatment-related mortality, and overall survival, of targeted sequential immune-depleting chemotherapy followed by reduced-intensity allogeneic HSCT from HLA-matched unrelated donors in the treatment of patients with advanced hematologic malignancies.

1.1.1.3 To determine and monitor incidence, organ severity and overall severity of chronic GVHD prospectively using the NIH Consensus Conference diagnosis and staging criteria and preliminarily validate those tools for use in clinical practice and trials (Pavletic, Imanguli, and Cowen).

1.1.2 Secondary Objectives:

1.1.2.1 To assess effects of two different GVHD prophylaxis regimens, TMS and AC, on CD4+ T cell receptor Vβ repertoire by CDR3 spectratyping at 1, 3, 6, and 12 months post-transplant (Hakim Lab).

1.1.2.2 To assess effects of two different GVHD prophylaxis regimens, TMS and AC, on CD8+ T cell receptor Vβ repertoire by CDR3 spectratyping at 1, 3, 6, and 12 months post-transplant (Hakim Lab).

1.1.2.3 To assess effects of two different GVHD prophylaxis regimens, TMS and AC, on the kinetics of CD4+ and CD8+ T-cells and NK cell depletion and recovery following the use of two different GVHD prophylaxis regimens, TMS and AC, in the setting of reduced-intensity allogeneic HSCT from HLA-matched unrelated donors (Hakim, Gress, and Hardy).

1.1.2.4 To correlate serum interleukin-7 and interleukin-15 levels during early immune reconstitution after two different GVHD prophylaxis regimens, TMS and AC, in the setting of sequential targeted immune-depleting chemotherapy followed by reduced-intensity allogeneic HSCT from HLA-matched unrelated donors (Mackall and Hakim).

1.1.2.5 To characterize the pattern of post-transplant $CD14^+$ monocyte production of inflammatory cytokines IL-1-α and TNF-α following the use of two different GVHD prophylaxis regimens, TMS and AC, in the setting of reduced-intensity allogeneic HSCT from HLA-matched unrelated donors (Hakim lab).

1.1.2.6 To assess the impact of two different GVHD prophylaxis regimens (TMS and AC) on donor/recipient chimerism in the setting of sequential targeted immune-depleting chemotherapy followed by reduced-intensity allogeneic HSCT from HLA-matched unrelated donors.

1.1.2.7 To determine the incidence and severity of acute GVHD following sequential targeted immune-depleting chemotherapy and reduced-intensity allogeneic HSCT from HLA-matched unrelated donors using two different GVHD prophylaxis regimens, TMS and AC.

1.1.2.8 To evaluate the feasibility of isolating and expanding clinically relevant numbers of tumor derived lymphocytes from patients' bone marrow or lymph nodes (Hardy).

1.1.2.9 To prospectively evaluate the pathogenesis of chronic GVHD by examining the immune cell reconstitution in the target tissues and cellular and molecular events preceding and coinciding with the clinical onset of chronic GVHD (Imanguli, Mays, Cowen, Gress, Hakim and Pavletic).

1.1.2.10 To prospectively assess long and short term cardiac toxicities of EPOCH-F in patients who have received greater than 450mg/m^2 of anthracyclines prior to study enrollment.

1.2 BACKGROUND AND RATIONALE

Allogeneic hematopoietic stem cell (a.k.a. bone marrow) transplantation (HSCT) is capable of producing sustained remissions in a number of hematologic malignancies. However, therapy-related toxicities, primarily related to the conditioning regimen and GVHD, and the availability of suitable allogeneic stem cell donors have limited the broader application of allogeneic HSCT.

1.2.1 Reduced-Intensity Conditioning Regimens in Allogeneic HSCT

Early studies of allogeneic HSCT utilized conditioning regimens containing high doses of alkylating agents often in combination with total body irradiation [1]. The use of such regimens, which are considered "myeloablative", was perceived as essential both for eradication of malignant disease and for suppression of the host-versus-graft response to the donor stem cells (i.e. rejection). These myeloablative conditioning regimens caused severe damage to normal organs within the body resulting in a high degree and incidence of patient morbidity and mortality [2]. This limited the application of allogeneic HSCT to younger (< 60 years) patients, who could withstand and recover from these toxicities. However, subsequent studies and observations challenged assumptions that myeloablative conditioning regimens were necessary to eliminate malignancy and to create the state of immunosuppression to permit the engraftment of the donor hematopoietic and immune systems within the recipient. The high frequency of relapse observed in the setting of T-cell-depleted allogeneic HSCT suggested that a significant proportion of the curative potential of allogeneic HSCT resulted from a T-cell-mediated graft-versus-tumor (GVT) [3]. The observation that delayed administration of donor lymphocytes

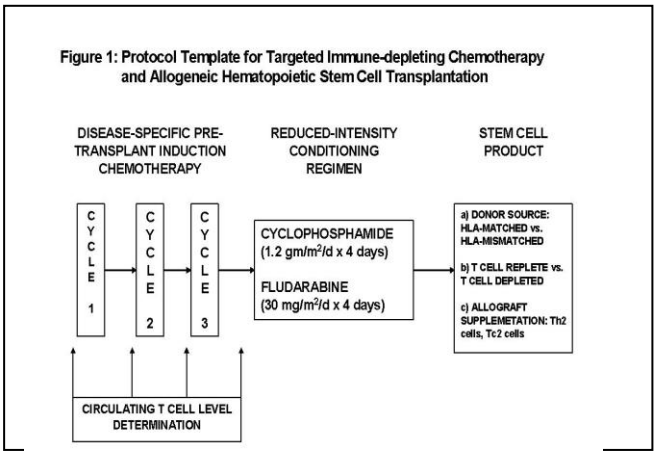
could induce complete remissions remote from the preparative regimen provided further evidence that an immune mechanism primarily mediates an anti-leukemic effects after allogeneic HSCT [4]. With respect to graft rejection, it is also now known that conventional myeloablative regimens do not consistently completely 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 allogeneic stem cell graft after myeloablative conditioning [5]. In addition, there are certain clinical situations where the malignancy is very susceptible to the allogeneic GVT effect (e.g. AML in complete remission, chronic leukemias or low-grade lymphomas). In these clinical situations the use of a myeloablative conditioning regimen may be causing significant toxicity without actually adding any clinical benefit in regard to elimination of the malignancy. These limitations of myeloablative conditioning regimens, combined with the high levels of morbidity and mortality associated with myeloablation, result in a low therapeutic index for this form of allogeneic HSCT. Reducing treatment-related toxicity would improve the therapeutic index of allogeneic HSCT, permitting the broader application and potential benefit of this treatment to more patients. This principle led to the development of less intensive ("non-myeloablative" and "reduced-intensity") conditioning regimens for allogeneic HSCT, which were designed with the purpose of allowing engraftment of donor cells to exploit the potential allogeneic GVT effect [6-10]. The early reports of studies employing non-myeloablative and reduced-intensity conditioning regimens prior to allogeneic HSCT demonstrated that engraftment occurred in the majority of patients, early (first 100 days after transplantation) transplant-related toxicities were reduced, and that this treatment could be applied to older patients and patients with co-morbidities. However, it was also observed that both non-myeloablative and reduced-intensity conditioning were associated with high incidences of incomplete donor chimerism, higher incidences of graft rejection/failure, and increased relapse rates, as compared to results with myeloablative conditioning regimens [11,12]. Mixed chimerism and graft rejection has been associated with HLA-disparity, use of unrelated donors, and inversely correlated with amount of therapy prior to transplant [13-15]. It was also observed that a GVT effect was not observed until full donor chimerism was achieved [16-19]. For this reason, a central hypothesis in the design of our transplantation protocols has been 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 Rationale for Targeted Immune Depletion Induction Prior to Reduced-Intensity Conditioning in the Setting of AlloHSCT from Unrelated Donors; Data from HLA-matched Siblings - CC 99-C-0143, CC 00-C-0119, and CC 03-C-0077

The objective to achieve rapid and complete donor chimerism following allogeneic HSCT led our group to adopt a strategy of sequential targeted immune depletion before transplant conditioning. Murine data from the Fowler Laboratory (NCI) demonstrated that daily administration of a combination of fludarabine and cyclophosphamide resulted in severe reductions in the number of circulating host $CD8^+$ and $CD4^+$ T cells to levels comparable to those achieved with myeloablative doses of total body irradiation [20]. This level of T cell depletion was sufficient to permit the engraftment of fully MHC-mismatched (i.e. haplo-identical) allografts (F1-into-parent murine model). The degree of myelosuppression with the extended administration of fludarabine and cyclophosphamide was significantly less than with total body irradiation (TBI). Taken together, these studies indicated that extended administration

of agents, which were highly immune-depleting yet had nominal myelosuppressive effects, could result in a level of host T cell depletion that would permit the engraftment of haplo-identical stem cells.

We used these preclinical murine data as the basis for our clinical trials design. It was our specific intent to design transplantation protocols that would rapidly result in complete donor chimerism, in order to optimize the GVT effect, since these protocols would be used to treat advanced malignancies. For many malignancies, however, the GVT effect may be inadequate by itself, and the cytotoxic effects of the conditioning regimen may therefore play a beneficial, if not necessary, role in the efficacy of allogeneic HSCT [21,22].



As such, we elected to employ a reduced-intensity conditioning regimen, consisting of fludarabine and cyclophosphamide, rather than a TBI-based non-myeloablative conditioning regimen. This reduced-intensity conditioning regimen has been utilized in all of our transplant protocols for clinical consistency and to permit comparison between protocols. Due to the variability of patients' immune status, we surmised that the level of host T cell depletion required to permit the rapid and complete engraftment of allogeneic stem cells would not be consistently achieved with the reduced-intensity conditioning regimen alone, especially if alternative donor stem cells (e.g. HLA-matched unrelated donors) and/or if T cell depletion of the allograft were to be utilized. To achieve uniform immune depletion, we elected to employ repetitive doses of immunosuppressive agents that had anti-tumor activity, as patients would require disease control prior to receiving a reduced-intensity conditioning regimen. However, we determined that the daily administration of these agents, as given in our murine model, was clinically impractical. As such, we elected to administer these agents at conventional doses in repetitive cycles, similar to how second-line or "salvage" regimens are given prior to autologous HSCT [23]. We refer to these as "induction regimens", as they have the two specific goals of inducing severe host T cell depletion as well as inducing disease remission. The most unique potential advantage is that the induction regimen would permit us to adjust or "target" the level of T cell depletion based upon a patient's pre- existing immune status. Specifically, the number of cycles of the induction regimen is determined by quantification of circulating host T cells after each cycle. In summary the rationale for incorporating "targeted immune-depletion" using induction chemotherapy in our protocol design (Figure 1) was that it: 1) allows us to achieve host T cell depletion in a patient-specific manner, 2) provides disease control, and 3) utilizes chemotherapy at doses with minimal levels of toxicity. The targeted immune-depletion approach has been applied to all of our allogeneic HSCT protocols.

We set out to evaluate our targeted immune-depleting approach in a series of sequential trials in which one of the factors that affects engraftment was altered in a step-wise fashion. In each successive study, the barriers to engraftment (e.g. T cell depletion, HLA-disparity) were increased, yet other engraftment variables (e.g. conditioning regimen and stem cell dose) were kept consistent to permit comparisons among protocols. Specifically in our first trial, 99-C-0143,

we investigated whether our targeted immune-depletion approach would result in rapid and complete donor chimerism in patients who received T cell replete (TCR; i.e. T cells were not removed from the allograft after collection) allografts from HLA-matched siblings. In 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. As a surrogate marker for the level of immune depletion necessary to achieve this goal, 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, would predict for robust engraftment after reduced-intensity allogeneic HSCT and be attainable with up to 3 cycles of induction chemotherapy before transplantation. Twenty patients were enrolled onto pilot arm 99-C-0143. In these 20 patients, the EPOCH-F regimen was very effective at reducing circulating T cell numbers, as it was observed that the goal of reducing the host circulating $CD4^+$ T cell numbers to less than 50 cells/μl was met after one to three cycles of this regimen in the majority of patients. All but one patient had a $CD4^+$ T cell value below 100 cells/μl after completing the last cycle of EPOCH-F. Nineteen patients received a TCR allograft from an HLA-matched sibling following the reduced-intensity conditioning regimen consisting of fludarabine and cyclophosphamide. Single agent cyclosporine was given as GVHD prophylaxis. Hematopoietic recovery was brisk with a median time to an absolute neutrophil count greater than 1.0×10^9/L of 10 days. All 19 patients were engrafted and there were no late graft failures. Evaluation of peripheral blood mononuclear cells for chimerism, as determined by a PCR-based typing of variable number tandem repeat (VNTR) polymorphisms for donor and host elements, demonstrated that 18 of 19 patients had greater than 95% donor cells by day +14 post-transplant. Subsequent analyses at days +28 and +100 post-transplant demonstrated that this level of engraftment was sustained. Subset chimerism analysis demonstrated that there was complete donor chimerism in both myeloid ($CD15^+$ or $CD33^+$) and T lymphoid ($CD3^+$) compartments, which were also maintained. Thus, with our approach of targeted immune-depletion we were able to achieve consistent, rapid, complete donor engraftment of TCR, HLA-matched related allogeneic stem cells. However, rapid and complete donor engraftment was also associated with a relatively high incidence (~70%) of clinically significant, defined as Grade II-IV, acute GVHD. Overall treatment-related mortality was acceptable at approximately 20% at one year post-transplant.

In light of these observations, in a subsequent trial, 03-C-0077, we employed a standard dual-agent GVHD prophylaxis regimen, cyclosporine + methotrexate, to determine the impact of this change on hematopoietic recovery, donor/recipient chimerism, and the incidence of acute GVHD. Methotrexate, in addition to its known anti-inflammatory activity, is believed to exert its immunosuppressive effects by inducing apoptosis and clonal deletion of activated T cells [24]. As such, the addition of methotrexate to our prior GVHD prophylaxis of single agent cyclosporine could have theoretically increased the frequency of mixed chimerism after transplantation, as well as the risk of graft rejection. The other transplant variables were otherwise kept the same, as patients again received TCR allografts from HLA-matched siblings. Thirty-one patients with advanced hematologic malignancies, primarily lymphomas were enrolled onto study and underwent allogeneic HSCT after targeted-immune depletion and

reduced-intensity conditioning (Bishop – unpublished data). All patients engrafted; there have been no late graft failures with a minimum of two years of follow-up. As had been hypothesized, there was a higher percentage (26%) of patients with early (day +14) mixed donor chimerism, defined as less than 95% donor chimerism; the median donor chimerism at this time point was 90% (range: 35-100%). However, by day+100 post-transplant 94% of patients had achieved full donor chimerism. The incidence of grade II-III acute GVHD was 42%, and there were no cases of Grade IV acute GVHD. The one- and two-year treatment-related mortality rates were 3% and 8%, respectively. At a minimum of two years of follow-up, 19 patients (61%) are alive of which 16 are in a complete remission at the time of this writing. Thus, this study demonstrated that the addition of methotrexate to the sequential targeted immune depletion approach still permitted rapid and complete donor engraftment in the majority of patients and resulted in a lower incidence of acute GVHD, improved treatment-related mortality, and beneficial overall outcomes in patients with high-risk hematologic malignancies.

1.2.3 Donor Availability for Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic HSCT is curative for many hematologic malignancies, but, as previously stated, stem cell donor availability limits the broader application of this treatment. Only 25 to 30% of patients have a sibling donor who is fully matched at the class I and II major histocompatibility complex (MHC) loci, which encode for the human leukocyte antigens (HLA-A, B, C, DRB1, DQB1, and DPB1). This limitation prompted the exploration of alternative stem cell sources for allogeneic HSCT including the use HLA-matched unrelated donors [25]. Enrollment of volunteer donors in national and international registries, such as National Marrow Donor Program (NMDP) in the United States, has facilitated the identification of unrelated stem cell donors for patients without HLA-matched sibling donors. However, transplants from unrelated donors are complicated by higher rates of graft rejection, acute and chronic GVHD, immune dysregulation, and treatment-related mortality. These complications are partially balanced by lower relapse rates attributed to increased histocompatibility differences. These complications have limited the application of myeloablative allogeneic HSCT from HLA-matched unrelated donors to patients less than 55 years of age and without any significant co-morbidities.

1.2.4 Effect of HLA on Transplant Outcomes*:*

Since its inception, the NMDP has required evaluation of donor-recipient histocompatibility matching (HLA-A, -B, and –DRB1) prior to unrelated donor HSCT [26]. The minimum acceptable match was originally defined by serologic testing at these 3 loci (6 possible antigens) and required at least 5 matches, that is, a “5 of 6 match”. This requirement has changed little over the years. Currently, to request a donor for transplantation, the minimal acceptable level of matching remains a 5 of 6 match for HLA-A, -B, and -DRB1. Although only evaluated at antigen level of resolution (“low resolution”) for donor release, each of these 3 loci must now be typed at high-resolution by DNA-based methods. High-resolution typing is defined as the identification of alleles based on differences in the antigen recognition site (ARS) domains (Exons 2 and 3 of Class I and exon 2 of Class II genes). In 2005, a requirement for HLA-C typing was added. The most recent studies have clearly shown that transplant outcomes can be improved by matching strategies that increase the overall degree of HLA compatibility above the minimum accepted level by matching also for HLA-C, -DPB1, -DQB1, and haplotypes [27].

As data on high resolution accumulated, the NMDP performed an analysis on the effects of HLA matching (low or high resolution or both) on engraftment, graft-versus-host disease (GVHD),

and mortality in 1874 donor-recipient pairs that were retrospectively typed using high resolution techniques for HLA-A, -B, -C, -DRB1, -DQB1, and –DPB1 [28]. There was no effect of any mismatch on engraftment, and only a mismatch at HLA-A affected whether the patients experienced Grade II-IV acute GVHD or chronic GVHD. Single mismatches at HLA-A, -B, -C, and -DRB1 each had similar adverse effects on mortality (relative risk 1.23 - 1.33), as compared to individuals matched at all four loci. The 5-year overall survival rates for a match at all four loci ("8 of 8" as compared to a single mismatch ("7 of 8") was 39% and 31%, respectively. In this analysis, mismatches at HLA-DQ or -DP did not appear to exert any significant effect on survival (5-year overall survival = 39%) or any other outcome. The observed adverse effects on outcome were more evident in transplants with low-resolution versus only high-resolution mismatches. When high-resolution mismatches at HLA-A, -B, -C, and -DRB1 were considered together, adverse effects on survival and GVHD were observed. The authors concluded that matching for HLA-C should be incorporated into algorithms for unrelated donor selection, along with HLA-A, -B, and -DRB1 (i.e. "8 of 8").

In a subsequent analysis, the NMDP looked at data from 3857 transplantations. Patient-donor pairs were fully typed for HLA-A, -B, -C, -DRB1, -DQB1, -DQA1, -DPB1, and -DPA1 alleles [29]. High-resolution DNA matching for HLA-A, -B, -C, and -DRB1 (8 of 8 match) was the minimum level of matching associated with the highest survival. A single mismatch detected by low- or high-resolution DNA testing at HLA-A, -B, -C or -DRB1 (7/8 match) was associated with higher mortality (relative risk = 1.25; 95% CI, 1.13-1.38; $P < 0.001$) and 1-year survival of 43% compared with 52% for 8 of 8 matched pairs. Single mismatches at HLA-B or HLA-C appeared to be better tolerated than mismatches at HLA-A or HLA-DRB1. Mismatching at 2 or more loci compounded the risk. Mismatching at HLA-DP or -DQ loci and donor factors other than HLA type were not associated with survival. In multivariate modeling, patient age, race, disease stage, and cytomegalovirus status were as predictive of survival as donor HLA matching. The authors concluded that high-resolution DNA matching for HLA-A, -B, -C, and -DRB1 alleles is associated with higher rates of survival.

Based on these results, the NMDP has established guidelines relative to donor selection based on HLA-typing [26]. The optimal donor should be matched at HLA-A, -B, -C, and -DRB1 by high-resolution typing. When there are multiple donors with this degree of matching, further matching at HLA-DQ may be utilized; however, the clinical utility does not reach statistical significance. If a mismatch is unavoidable, a single mismatch at HLA-A, -B, -C, and -DRB1 should be sought. In the NMDP data, mismatches at HLA-B and -C may be less detrimental than those at HLA-A and -DRB1.

1.2.5 Experience with Non-myeloablative and Reduced-Intensity Conditioning Regimens from Matched Unrelated Donors:

In light of the high treatment-related morbidity and mortality associated with myeloablative allogeneic HSCT from unrelated donors, clinical studies utilizing non-myeloablative and reduced-intensity conditioning regimens were initiated after encouraging results with these regimens for HLA-matched siblings had been reported. However, because of greater genetic disparity between the donors and recipients, it was hypothesized that non-myeloablative conditioning regimens for unrelated donor transplantation might require more intense immunosuppression than a transplant from an HLA-matched sibling. Since 2001, there have been a number of relatively large single-institution and multi-institutional studies on non-

myeloablative and reduced-intensity allogeneic HSCT utilizing unrelated donors (Tables 2A and 2B) [14,30-34]. It is of significant note that the median ages of recipients have ranged from 44 to 59 years with patients as old as 72 years successfully undergoing this procedure. As had been hypothesized the incidence of graft rejection, ranging from 0 to 21%, was increased. The primary adverse factors related to graft rejection were related to use of non-myeloablative (as opposed to reduced-intensity) conditioning regimens, HLA-mismatching, use of bone marrow rather than mobilized peripheral blood, and a minimal amount of prior therapy prior to transplant. In particular, the incidence of graft rejection was as high as 44% among patients who received unrelated bone marrow after a TBI-based non-myeloablative conditioning regimen in the experience of the Fred Hutchinson Cancer Research Center [14]. Using a variety of GVHD prophylaxis regimens, the reported incidences of Grade II-IV and Grade III-IV acute GVHD have been 21-63% and 6-39%, respectively. The lowest acute GVHD rates have been achieved with regimens employing in vivo T cell depletion with anti-T cell monoclonal antibodies, particularly alemtuzumab [31]. This latter approach has also been associated with increased late rejections and opportunistic infections. The reported incidence of chronic GVHD have varied from 8% to 68% with the lowest rates reported with the use of alemtuzumab. Early ($\leq$ 100 days post-transplant) treatment-related mortality rates have varied from 10 to 48%; one-year treatment-related mortality rates have varied from 17 to 55%. One-year overall survival rates have been reported to be 32 to 75%.

Table 2A - Patient and Treatment Characteristics of Reported Trials of Non-myeloablative and Reduced-intensity Allogeneic HSCT Utilizing HLA-matched Unrelated Donors

Author/Institute Journal/Date	Patient # Median Age	HLA Loci	Degree match	Level of HLA Match		Conditioning Regimen	GVHD Prophylaxis	Stem Cell Source	CD34 Dose (10^6/kg)	CD3 Dose (10^8/kg)
				Class I	Class II					
Giralt et al. MDACC Blood 2001 [Ref #30]	N = 86 (URD = 40) 52 years (22-70)	6	6/6	Serologic	Molecular	Flu (25 mg/m^2/d) x 5d Mel 90–140 mg/m^2 Clad (12 mg/m^2/d) x 5d Mel 90–140 mg/m^2	FK506 x 6 months MTX (5 mg/m^2) +1,+3,+6,+11	NA	NA	NA
Chakraverty et al. UK multi-institute Blood 2002 [Ref #31]	N = 47 44 years (18–62)	10	9-10/10 (s) 8-10/10 (m)	serologic – A,B molecular – C	Molecular	Flu (30 mg/m^2/d) x 5d Mel 140 mg/m^2 CAMPATH (20 mg/kg) -8,-7,-6,-5,-4,-3	CsA x 3 months [CAMPATH]	BM = 46 PB = 1	NA	NA
Kroger et al. German multi-institute Blood 2002 [Ref #32]	N = 21 (all multiple myeloma) 50 years (32-61)	8 HLA-A,B, DRB1,DQB1	7-8/8	Serologic	Molecular	Flu (30 mg/m^2/d) x 5d Mel 100 mg/m^2 ATG (10 mg/kg) -3,-2,-1	CsA x 3 months MTX (10 mg/m^2) +1,+3,+6	BM = 6 PB = 15	3.9 (0.4 - 12.5)	NA
Niederwieser et al. FHCRC Consortium Blood 2003 [Ref #33]	N = 52 48 years (6–65)	8 (10) HLA-A,B,C, DRB1, (DQB1)	7-8/8 (s) 6-8/8 (m)	Serologic	Molecular	Flu (30 mg/m^2/d) x 3d TBI 200 cGy	CsA x 2-3 months, taper until +180) MMF x 40d,taper	BM = 13 PB = 39	6.0 (1.5 – 21.6) (BM = 2.7)	3.4 (0.8 – 10.0) (BM = 0.3)
Maris et al. FHCRC Consortium Blood 2003 [Ref #14]	N = 85 53 years (5–69)	10 HLA-A,B,C, DRB1,DQB1	10/10	serologic (81% molecular at Class I)	Molecular	Flu (30 mg/m^2/d) x 3d TBI 200 cGy	CsA x 3 months, taper until +180 MMF x 40d,taper	BM = 18 PB = 71	6.99 (1.26 -16.4)	2.61 (0.8 -37.7)
Wong et al. MDACC Blood 2003 [Ref #34]	N = 29 59 years (55-69)	6	6/6 5/6 (n = 2)	Serologic	Molecular	Flu (25 mg/m^2/d) x 5d Mel 90–140 mg/m^2 +/- ATG (n = 17)	FK506 x 6 - 8 months MTX (5 mg/m^2) +1,+3,+6,+11	BM = 28 PB = 1	4.46 (0.37 – 8.6)	NA

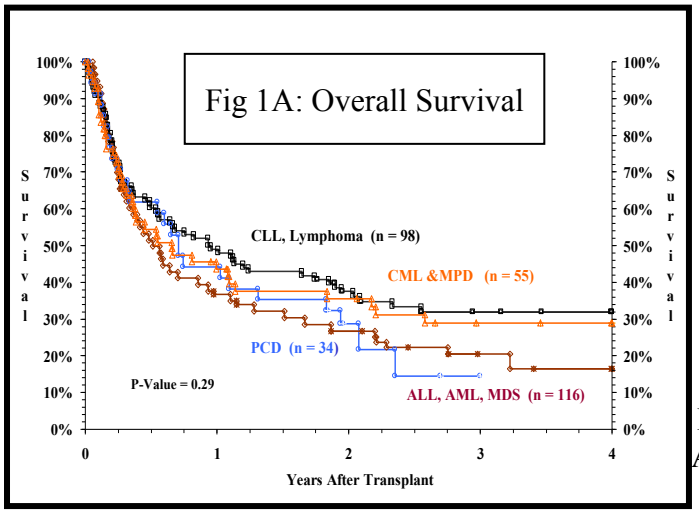
Table 2B. Clinical Outcomes of Reported Trials of Non-myeloablative and Reduced-intensity Allogeneic HSCT Utilizing HLA-matched Unrelated Donors

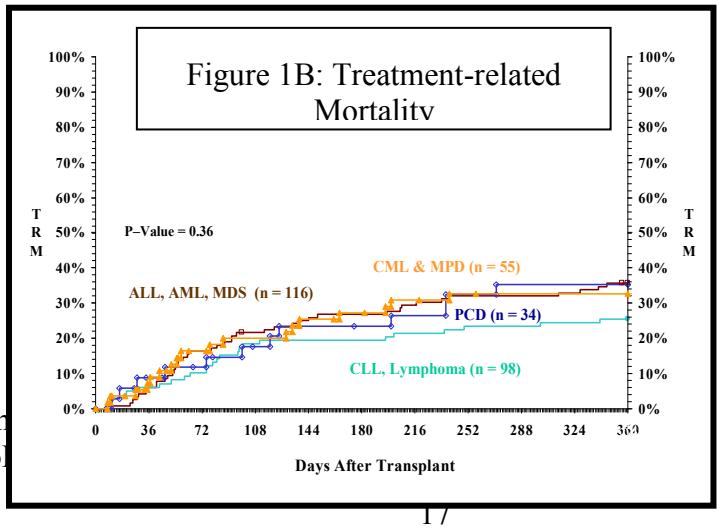
Author/Institute Journal/Date	Graft Rejection and Failure	Acute GVHD		Chronic GVHD	TRM			OAS	
		II-IV	III-IV		Day 100	1 year	2 year	1 year	2 year
Giralt et al. MDACC Blood 2001	5% (n =2)	62% (11 deaths)	39%	68%	37.4%	NA	45% (all patients)	32% (URD)	28% (all patients)

Chakraverty et al. UK multi-institute Blood 2002	3% (n= 2)	21.3%	6.4%	8% (all limited)	15%	20%	(~25%)	75%	NA
Kroger et al. German multi-institute Blood 2002	0	38%	19%	37% Limited = 25% Extensive = 12%	10%	26%	NA	74%	74%
Niederwieser et al. FHCRC Consortium Blood 2003	12% (low CD3; low CD34)	63%	21%	60% Limited = 30% Extensive = 30%	11%	29%	40%	44%	35% (19 mo)
Maris et al. FHCRC Consortium Blood 2003	21% (PB vs. BM: 15% vs. 44%)	52%	10%	45% (85) Extensive = 37%	11%	17%	NA	52%	(~42%)
Wong et al. MDACC Blood 2003	14% (n= 4)	41%	20%	62.5%	48%	55%	NA	44%	(~44%)

In regard to specific disease outcomes, data (Figure 2A) from the Center for International Blood and Marrow Transplant Research (CIBMTR) demonstrate two-year overall survival rates varying between approximately 25-40% depending of the specific hematologic malignancy. CIBMTR data (Figure 2B) on one-year treatment mortality rates (26-36%) were consistent with reports from single-institution and multi-institutional studies. These data demonstrated that the use of non-myeloablative and reduced-intensity conditioning regimens could successfully be performed in older patient; however, there is a need for significant correlative studies and clinical improvements relative to engraftment, reductions in GVHD, immune reconstitution, treatment-related mortality, and overall survival, as a clearly superior approach to this treatment has yet to emerge.

Figures 2A and 2B: CIBMTR Analysis of Overall Survival and Treatment-related Mortality after Non-Ablative or Reduced-Intensity HSCT with Unrelated Donors





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As described above, the use of a standard dual-agent GVHD prophylaxis regimen, cyclosporine + methotrexate, in 03-C-0077 permitted relative rapid and complete donor engraftment and resulted in an acceptable incidence of clinically significant GVHD and a relatively low overall treatment-related mortality following our approach of targeted immune depletion prior to reduced-intensity conditioning in the setting of allogeneic HSCT from HLA-matched siblings. Similar calcineurin-inhibitor-based GVHD prophylaxis regimens (i.e. FK506)/methotrexate) have been investigated in the setting of reduced-intensity allogeneic stem cell transplantation utilizing unrelated donors [30]. They have been associated with relatively higher rates of GVHD, as compared to other regimens that have been employed, particularly alemtuzumab (Table 2B). It is predicted that the combination of cyclosporine + methotrexate will be inadequately immunosuppressive to prevent clinically severe (grade III-IV) acute GVHD if our targeted immune depletion approach were employed in the setting of reduced-intensity allogeneic HSCT from unrelated donors.

The two regimens which have been reported to be most successful at preventing acute GVHD following reduced-intensity allogeneic HSCT from unrelated donors have been the combination of alemtuzumab and cyclosporine [31] and the combination of tacrolimus, methotrexate, and sirolimus [36]. Both regimens have distinct clinical advantages and disadvantages, as well as distinct mechanisms of action.

1.2.7 Tacrolimus/Methotrexate/Sirolimus as Graft-Versus-Host Disease Prophylaxis

More recently, the immunosuppressive drug sirolimus, which is effective in the prevention of renal allograft rejection [31], has been evaluated as a third agent for GVHD prophylaxis after allogeneic HSCT from unrelated donors [32]. Sirolimus uniquely inhibits T cell and dendritic cell function through inhibition of mTOR, which in contrast to the mRNA inhibition associated with calcineurin blockade, operates via a post-transcriptional mechanism to reduce cell signaling and modulate protein translation and phosphorylation in T cells [37]. Clinical studies have not evaluated single-agent sirolimus for GVHD prophylaxis, and in fact, murine data indicate that single-agent sirolimus potently abrogates CD8+ T cell mediated GVHD, but not CD4+ T cell mediated GVHD [38]. As such, sirolimus is likely to represent an adjunct to, but not a replacement of, calcineurin inhibitor mediated GVHD prophylaxis. Initial results indicate that addition of sirolimus to GVHD prophylaxis is effective for the prevention of acute GVHD. Investigators at the Dana-Farber Cancer Institute (DFCI) performed a single arm study, which involved unrelated donor or mismatched family member bone marrow transplantation, the GVHD prophylaxis regimen consisted of calcineurin-inhibitor tacrolimus, methotrexate, and sirolimus [36]. Sirolimus was initiated at a loading dose of 12 mg, with a maintenance dose of 4 mg per day throughout the first 100 days post-transplant. The incidence of grade II-IV acute GVHD associated with this regimen was 26% (11/41), which is reduced relative to historical controls previously described above. Sirolimus was also reported to be well-tolerated at this dosing schedule, with hyper-triglyceridemia representing the primary specific drug toxicity.

Sirolimus has been of significant interest and investigation in the Fowler Laboratory. In an ongoing clinical protocol, 04-C-0055, short-course sirolimus administration (fourteen day treatment interval) has been evaluated in combination with cyclosporine with and without Th2 cells as GVHD prophylaxis in the setting of non-myeloablative targeted immune depletion and TCR allogeneic HSCT from HLA-matched siblings. The Fowler Laboratory hypothesized that short-course *in vivo* sirolimus would preferentially inhibit unmanipulated donor T cells contained

within the allograft, thereby resulting in preferential *in vivo* expansion of sirolimus generated Th2 cells. However, it is also possible that sirolimus alone (i.e. without Th2 cells) would contribute to GVHD prophylaxis, as murine allogeneic bone marrow transplantation experiments in the Fowler Laboratory demonstrated that short-course sirolimus administration in the peri-transplant period significantly reduced fully-MHC mismatched graft-versus-host reaction. Preliminary results from 04-C-0055 suggest that the arm (III) of this trial receiving only cyclosporine and sirolimus (i.e. no Th2 cells) has resulted in an incidence of acute GVHD similar to that achieved with cyclosporine + methotrexate. In the first 11 evaluable patients on arm III of 04-C-0055, there have only been two case acute GVHD, which were grade I and grade II, respectively (*D. Fowler - personal communication*).

1.2.8 Alemtuzumab/Cyclosporine as Graft-Versus-Host Disease Prophylaxis:

Alemtuzumab is a humanized monoclonal antibody that targets the CD52 antigen, which is highly expressed on both B and T lymphocytes, and has a relatively long half-life that is measured in weeks. Alemtuzumab has been utilized, both ex-vivo and in vivo, to prevent GVHD in individuals undergoing allogeneic HSCT from unrelated donors for over 20 years [39]. More recently has alemtuzumab has been utilized as part of non-myeloablative conditioning regimens. Alemtuzumab has been administered along with non-myeloablative and reduced-intensity conditioning regimens to provide pan-depletion of both host and donor B- and T-cells [31,40-42]. These actions may promote engraftment through the depletion of host T cells and prevent GVHD through the in vivo T cell depletion of the allograft.

Kottardis and colleagues reported on their use of alemtuzumab in high-risk 44 patients with hematologic malignancies undergoing non-myeloablative allogeneic HSCT [36]. The conditioning regimen consisted of alemtuzumab (20 mg/day on days -8 to -4, fludarabine (30 mg/m^2 on days -7 to -3), and melphalan (140 mg/m^2 on day -2). Thirty-six recipients received unmanipulated G-CSF mobilized peripheral blood stem cells from HLA-identical siblings, and 8 received unmanipulated marrow from matched unrelated donors. Additional GVHD prophylaxis consisted of cyclosporine alone for 38 patients and cyclosporine plus methotrexate for 6 sibling recipients. Forty-two of the 43 evaluable patients had sustained engraftment. Results of chimerism analysis demonstrated that 18 of 31 patients studied were full-donor chimeras, while the other patients were mixed chimeras in one or more lineages. There were no cases of grades III-IV acute GVHD. Only 2 patients developed grade II acute GVHD, and only 1 had chronic GVHD. The estimated probability of non-relapse mortality was 11%. Four patients died of regimen-related complications. At a median follow-up of 9 months (range: 3 - 29 months), 33 patients were alive in complete remission or with no evidence of disease progression. Seven patients relapsed or progressed post-transplantation, and four of them subsequently died.

Chakraverty reported on 47 patients with hematological malignancy who underwent allogeneic HSCT from HLA-matched, unrelated donors following a non-myeloablative conditioning regimen containing alemtuzumab [31]. Additional GVHD prophylaxis consisted of cyclosporine alone. The median patient age was 44 years. Twenty of the transplants were mismatched for HLA class I and/or class II alleles. Primary graft failure occurred in only 2 of 44 evaluable patients (4.5%). Chimerism studies in 34 patients indicated that the majority (85.3%) attained initial full donor chimerism. Only 3 patients developed grade III to IV acute GVHD, and no patients had developed chronic extensive GVHD at the time of this report. The estimated probability of non-relapse mortality at day +100 post-transplant was 14.9%. At a median follow-

up of 344 days (range: 79-830), the overall and progression-free survival rates at 1 year were 75.5% and 61.5%, respectively.

The Spanish and United Kingdom Collaborative Groups for Nonmyeloablative Transplantation investigated the efficacy of alemtuzumab as compared with methotrexate for GVHD prophylaxis in 129 recipients of a non-myeloablative allogeneic HSCT from an HLA-matched sibling enrolled in 2 prospective studies for chronic lymphoproliferative disorders [41]. The conditioning regimens in both studies were based on the same combination of fludarabine and melphalan, but the United Kingdom (UK) study utilized a GVHD regimen consisting of cyclosporine plus alemtuzumab, whereas the Spanish study used cyclosporine plus methotrexate for GVHD prophylaxis. Patients receiving alemtuzumab had a higher incidence of cytomegalovirus (CMV) reactivation (85% vs. 24%, $p < 0.001$) and a significantly lower incidence of acute GVHD (21.7% vs. 45.1%, $p = 0.006$) and chronic GVHD (5% vs. 66.7%, $p < 0.001$). Twenty-one percent of patients in UK study group and 67.5% in Spanish study group had complete or partial responses three months after transplantation ($p < 0.001$). Eighteen patients in the UK study group received donor lymphocyte infusions to achieve disease control. There was no difference in disease status between the groups with 71% versus 67.5% ($p = 0.43$) of patients showing complete or partial responses in the UK and Spanish groups, respectively. No significant differences were observed in event-free or overall survival between the two groups.

The use of alemtuzumab in the non-myeloablative transplant setting has been complicated with a relative high incidence of cytomegalovirus (CMV) re-activation and infections [42,43]. CMV was monitored by polymerase chain reaction-based assays and treated preemptively in 101 patients who underwent allogeneic HSCT following a non-myeloablative conditioning containing alemtuzumab [39]. Fifty-one patients (50%) were observed to have CMV re-activation at a median of 27 days after transplantation. All three patients who developed clinical CMV disease died of this complication in this series. The median time to CD4+ T-cell count more than 200/microL was 9 months in the 48 patients studied. The probabilities of overall survival and non-relapse mortality at 18 months were 65% and 27.8%, respectively, with no significant difference in survival between patients with and without CMV infection.

1.2.9 Ex vivo T-cell Depletion in Non-myeloablative and Reduced-intensity Allogeneic HSCT

Relative to other methods of preventing acute GVHD, there have been reports on the use of ex vivo T cell depletion following myeloablative allogeneic HSCT from unrelated donors, beginning with the first reports on the use unrelated donors [40,41]. Ex vivo T cell depletion resulted in decreased acute GVHD, but it was associated with increased graft failure and opportunistic infections resulting in no clear survival advantage over unmanipulated grafts in the myeloablative setting. This led to a phase III multi-institutional trial in which 405 patients were randomized to either ex-vivo T-cell depletion of the marrow with cyclosporine alone (n = 201) versus an unmanipulated marrow with methotrexate and cyclosporine (n = 204) as GVHD prophylaxis [42]. The primary outcome was 3-year disease-free survival, which was analyzed by intention to treat. Disease-free survival at 3 years was 27% and 34% in recipients of T cell deplete arm and the methotrexate and cyclosporine arm, respectively ($p = 0.16$). T cell depletion was associated with significantly more rapid neutrophil recovery (15 days vs. 20 days, $p < 0.0001$), less grade III-IV acute GVHD (18% vs. 37%, $p < 0.0001$), reduced grade III-IV toxicities (19% vs. 29%, $p = 0.017$), reduced duration of initial hospitalization, but a higher risk of chronic myelogenous leukemia relapse (20%vs 7%, p=0.009) and CMV infection (28% vs.

17%, p = 0.023) than patients receiving methotrexate and cyclosporine. In a follow-up paper to this trial, the impact of ex vivo T cell depletion on chronic GVHD was analyzed by Pavletic and collaborators [47]. The incidence of chronic GVHD at 2 years was similar between the two arms (T cell depletion = 29% vs. methotrexate/cyclosporine = 34%, p = 0.27). Survival at 3 years from diagnosis of chronic GVHD was also similar (T cell depletion = 51% vs. methotrexate/cyclosporine = 58%; p = 0.29). The incidence of serious infections and leukemia relapse were similar on both treatment arms. In spite of a significant reduction of acute GVHD, T cell depletion did not reduce the incidence of chronic GVHD or improve survival in patients who developed chronic GVHD. There have only been anecdotal reports on the use of ex vivo T cell depletion after either non-myeloablative or reduced-intensity conditioning regimens utilizing either HLA-matched or unrelated donors. One of the larger experiences came from the National Cancer Institute [48]. In this trial, planned, sequential donor lymphocyte infusions given early (day +42) post-transplant resulted in an incidence of acute GHVD similar to that observed with a T cell replete allograft.

1.3 PRIMARY AIMS

1.3.1 Assessment of immune reconstitution following reduced-intensity allogeneic HSCT from unrelated donors.

Immune reconstitution following allogeneic HSCT is a prolonged process, influenced by a number of factors including, the age and corresponding thymic function of the transplant recipient, and degree histocompatibility between the donor the recipient, as well as the presence or absence of GVHD. However, immune reconstitution has not been well studied in the setting of reduced-intensity allogeneic HSCT from unrelated donors. It is our interpretation of the available medical literature that both of the AC and TMS GVHD prophylaxis regimens are effective in preventing acute GVHD in the setting of reduced-intensity allogeneic HSCT using HLA-matched unrelated donors. However, due to their distinctive biologic differences, it is hypothesized that these two regimens have significantly different effects upon immune reconstitution, which has significant implications to other factors affecting outcomes after transplantation (e.g. infections and relapse). A detailed, prospective analysis on the effects of either of these regimens on immune reconstitution has not been performed, and such an analysis is warranted relative to both transplant biology and clinical outcomes. It is our intent to assess and compare in a pilot manner immune reconstitution following the combination of alemtuzumab and cyclosporine, as employed by the United Kingdom Collaborative Groups for Nonmyeloablative Transplantation group [31] and the combination of tacrolimus, methotrexate, and sirolimus, developed at DFCI [36], in the setting of reduced-intensity allogeneic HSCT from HLA-matched unrelated donors.

Immune reconstitution will be assessed in a variety of techniques with which we and our collaborators are recognized for specific expertise. One assessment of immune reconstitution will be an analysis of T-Cell Receptor Vβ repertoire using CDR3 spectratyping. The complementarity-determining region (CDR3) of the T-cell receptor (TCR) is of most importance for the extensive diversity of the TCR [49,50]. The CDR3 plays a key role in defining the specificity of antigen recognition because the region forms the contact site for binding to peptide major histocompatibility complexes (MHCs) expressed by antigen-presenting cells. The CDR3 of both TCRα and TCRβ genes is generated by extensive rearrangement and fusion between the V, D, and J segments and by random insertion and deletion of junctional nucleotides, which yields final products that are quite heterogeneous in size. As a result of these gene

rearrangements, each T cell has a unique TCR and the diversity of the T-cell repertoire at any specific time can be characterized by the examination of CDR3 within that population. Healthy individuals demonstrate a diverse and polyclonal TCR repertoire reflected by a Gaussian size distribution of CDR3 regions composed from the TCR Vβ gene [47]. Amplification of the Vβ region expressed in circulating T cells has been used by the technique called CDR3 spectratyping to monitor the Vβ gene expression as a measurement of the level of immune competence in allogeneic HSCT recipients in both the myeloablative and reduced-intensity settings [52,53]. Factors such as T cell depletion, the development of GVHD, high recipient age, and infections are known to impair immune competence after allogeneic HSCT and correlate to oligoclonal or monoclonal CDR3 patterns [54-56]. Different CDR3 sizes and a Gaussian distribution of these peaks should be present in a normal polyclonal expression of a Vβ subfamily [51]. These differences can be quantified and compared. The overall complexity within a Vβ subfamily can be determined by counting the number of discrete peaks per subfamily. Subfamilies can then be graded on a score based on the degree of complexity. Normal complexity is characterized by a Gaussian distribution of transcript sizes, reflecting the presence of polyclonal cDNA species. CDR3 spectratype analysis provides a sensitive method for characterization of the T-cell repertoire as well as for following changes in individual patients over time. CDR3 spectratype analysis is an ideal method to assess differences in immune reconstitution after the administration of these two regimens, which have distinct effects upon T cells over time post-transplant.

As part of the comprehensive primary objective of assessing the effects of these two GVHD regimens on immune reconstitution, we will specifically assess differences in CD4+ T cell receptor Vβ repertoire by CDR3 spectratyping at 3 months post-transplant. This specific T cell subset was selected as this population generally recovers relative to its effects on other lymphocyte populations (i.e. CD8+ T cells and B lymphocytes). In addition, the homeostatic mechanisms which influence CD4+ T cell reconstitution (i.e. IL-7) are less perturbed by other host environmental factors. We chose three months post-transplant as this is time point at which repertoire diversity previously has been demonstrated to increase. Three months is also the time point at which scheduled tapering of immunosuppression is planned and clinical assessments and decisions are made relative to GVHD and disease status. These data will be complemented by spectratype analysis on both CD4+ and CD8+ T cells at various time points as well as the additional data on immune reconstitution which will be performed as part of the secondary aims. This extensive and extremely unique data set on immune reconstitution will be of value to the general transplantation field and in the design of our future protocols utilizing unrelated donors.

1.3.2 Assessment of chronic GVHD in the setting of sequential targeted immune-depleting chemotherapy followed by reduced-intensity allogeneic HSCT from HLA-matched unrelated donors

In addition to acute GVHD, chronic GVHD contributes to the morbidity and mortality associated with allogeneic HSCT from unrelated donors [56]. Chronic GVHD has not been well characterized in either the non-myeloablative and reduced-intensity allogeneic HSCT settings, particularly related to the use of unrelated donors. The reported incidences of chronic GVHD following non-myeloablative and reduced-intensity allogeneic HSCT from unrelated donors has varied from 8 – 65% (Table 2B), depending on patient age, degree of HLA-matching, and form of GVHD prophylaxis [14,30-34]. Chronic GVHD has become a specific area of intent research within ETIB. Led by Dr. Steven Pavletic, the Chronic Graft-versus-Host Disease Program is a

multi-institutional and extramural collaborative effort which incorporates laboratory and clinical studies including the establishment of one of the world's largest multi-disciplinary clinic for chronic GVHD, utilizing the unique resources and expertise available at the NIH. The efforts by Dr. Pavletic and his NIH collaborators were highlighted by the 2005 NIH-sponsored Chronic GVHD Consensus Project, which has unified the transplant community's approach to chronic GVHD through the activities of focused working groups [61-63]. Patients will be specifically monitored within the Chronic GVHD Clinic, will be offered potential participation on the Chronic GVHD Natural History protocol (04-C-0281), and will potentially be offered participation on therapeutic trials which are currently submitted for review and under development in the NIH. (Please see section **1.6**)

1.4 Background on Secondary Objectives

1.4.1 Assessment of serum Interleukin-7 and Interleukin-15 following Reduced-Intensity Allogeneic HSCT from Unrelated Donors

Immune reconstitution has been a primary research focus of both the Gress and Mackall Laboratories, both of whom are associate investigators on this study [57-61]. Secondary endpoints will continue according to prior studies performed as part of 99-C-0143, 00-C-0119, and 03-C-0077. These studies have included the kinetics of CD4+ and CD8+ T-cells and NK cell depletion and recovery in the setting of sequential immune depletion and reduced-intensity allogeneic HSCT. They have also included, as part of 03-C-0077, the correlation of serum IL-7 and IL-15 levels with lymphocyte subpopulations prior to transplantation and during immune recovery after transplant, and during the development of clinical GVHD, which had never been determined previously in the clinical setting. Preliminary analysis of data from 03-C-0077 demonstrates that there is an association of post-transplant serum IL-7 levels with the development of acute GVHD. The continuation of these studies in the setting of allogeneic HSCT from unrelated donors will potentially provide valuable insights relative to immune reconstitution as it relates to post-transplant toxicities, particularly GVHD and immune dysregulation.

1.4.2 Characterization of post-transplant monocyte production of inflammatory cytokines IL-1-α and TNF-α after reduced-intensity allogeneic HSCT

Characterization of the inflammatory cytokine cascade after allogeneic HSCT, aptly termed "cytokine storm" GVHD [60], has identified IL-1-α and TNF-α as potential targets for GVHD prevention or therapy. Indeed, neutralization of these cytokines can reduce murine gut GVHD [61,62], and importantly, this approach to GVHD prevention can be accomplished without abrogating an allogeneic T cell mediated graft-versus-leukemia (GVL) effect [67,68].

The existence of a similar non-T cell mediated inflammatory cytokine phase of GVHD in human transplantation has been more difficult to prove. Given the role of the macrophage as cell source for inflammatory cytokine-mediated murine GVHD, Fowler and colleagues reasoned that clinical "cytokine storm" might be monitored in a sensitive manner through sequential post-transplant evaluation of circulating monocyte production of IL-1-α and TNF-α using an intracellular flow cytometry assay [65]. Seventeen patients with refractory hematologic malignancy received reduced-intensity conditioning, HLA-matched sibling HSCT, and GVHD prophylaxis consisting of cyclosporine alone. After HSCT, monocyte IL-1-α and TNF-α were measured by intracellular flow cytometry (IC-FCM), and results were correlated with acute

GVHD. Post-transplant monocyte IL-1-α increased significantly (p = 0.0065) from week 2 to week 4 post-transplant; however, it was not associated with GVHD severity (p = 1.00). In contrast, increases in monocyte TNF-α were quantitatively reduced and temporally delayed, from week 2 to week 6 (p = 0.076). Elevation of monocyte TNF-α correlated with increased gut GVHD severity (p = 0.0041), with increases in monocyte TNF-α typically preceding the onset of gut GVHD symptoms. Serial measurement of monocyte cytokines, in particular, TNF-α, by IC-FCM represents a non-invasive method for GVHD monitoring, potentially allowing identification of patients appropriate for early intervention strategies.

1.4.3 Tumor derived lymphocytes

The prognosis for most patients with hematologic malignancies that have persistent disease or relapse after allogeneic HSCT is poor. Effective therapy for patients who fail withdrawal of immune suppression and administration of donor lymphocyte infusions (DLI) has not been identified. Even when DLI are effective, the response is often not durable and can be accompanied by GVHD. Generation of donor lymphocytes that mediate more potent and specific GVT effects while minimizing GVHD is a major laboratory and clinical effort of the ETIB. We have hypothesized that lymphocytes found in tumor after alloHSCT are of donor origin, and because they are tumor-derived, they may be tumor-specific in their homing and antigen specificity characteristics. In preclinical studies we have developed a method for the activation and expansion of tumor-derived lymphocytes (TDL) through CD3/CD28 co-stimulation and further expansion in IL-2. This approach is being piloted in patients with B cell malignancies who have persistent disease or relapse after AlloHSCT, including recipients of URD allografts. In parallel, ongoing preclinical efforts are focusing on optimization of the TDL cell product development, including cell yields and tumor specificity. Research samples obtained from subjects enrolled on this protocol will provide valuable data that may be of benefit to URD allograft recipients for whom DLI are not always readily available.

1.5 SUMMARY

The major limitations to the broader applicability of allogeneic HSCT for the treatment of malignancies are lack of suitable donor and therapy related toxicities. The use of stem cells from unrelated donors partially alleviates the need for donors, but their use is associated with a higher incidence of graft rejection and mixed donor chimerism, due to histocompatibility difference between the donor cells and the recipient. Based on the theory that the rapid establishment of donor chimerism was essential for an optimal GVT effect, we have employed a strategy of targeted immune-depleting chemotherapy prior to reduced-intensity allogeneic HSCT in a series of sequential trials in which one of the factors that affects engraftment was altered in a step-wise fashion. A recently completed trial, 03-C-0077, demonstrated that targeted immune depletion permitted rapid and complete donor engraftment, a lower incidence of acute GVHD, improved treatment-related mortality, and beneficial overall outcomes in patients with high-risk hematologic malignancies who received a reduced-intensity allogeneic T cell replete HSCT from HLA-matched sibling, using a GVHD prophylaxis regimen consisting of cyclosporine and methotrexate. It is our intent to investigate this approach in the setting of HLA-matched unrelated donors.

A clearly superior GVHD prophylaxis regimen has not been established in the unrelated donor setting. The two GVHD prophylaxis regimens with which best results have been reported are alemtuzumab with cyclosporine [31] and the combination of tacrolimus, methotrexate, and

sirolimus [36]. Both of these regimens are likely to provide adequate prophylaxis against the development of clinically severe acute GVHD in the setting of targeted immune-depleting chemotherapy prior to reduced-intensity allogeneic HSCT from HLA-matched unrelated donors. However, the two regimens work through biologically distinct mechanisms, and their effects upon immune reconstitution, which have not previously been well studied with either regimen, are predicted to be markedly different, regardless of the presence or absence of acute GVHD.

The study design provides a unique opportunity to investigate and compare in a preliminary fashion post-transplant immune reconstitution related to each of these GVHD prophylaxis regimens, which have distinct biologic properties relative to their ability to prevent GVHD. Immune reconstitution will be determined by a variety of techniques with an emphasis on CDR3 spectratyping. There has not been a report on post-transplant immune reconstitution in the available medical literature, as extensive or prospectively performed, as the studies planned in this protocol. These studies are a natural extension of our prior work and take advantage of the unique expertise and resources within the laboratories of the ETIB, intramural programs of the NCI, and the internationally recognized expertise on CDR3 spectratyping at Hackensack University with whom we have formed a formal collaboration. This study will provide preliminary data relative to multiple aspects of immune reconstitution after allogeneic HSCT from unrelated donors, which is important and essential to the understanding of this biology and development of strategies to improve the most important complications after this procedure, specifically GVHD and immune dysregulation.

Although both of these GVHD regimens are likely to provide adequate prophylaxis against the development of clinically severe acute GVHD in the setting of targeted immune-depleting chemotherapy prior to reduced-intensity allogeneic HSCT from HLA-matched unrelated donors, this needs to be documented, as well as other clinical endpoints relative to the safety of the procedure. To this end, we will assess, as a primary objective, engraftment, early and late treatment-related mortality, and overall survival and have established stringent stopping rules for each parameter.

The results of this pilot trial will be used for the development of subsequent studies related to allogeneic HSCT from unrelated donors incorporating techniques and approaches that are currently under investigation in the ETIB and POB.

1.6 AMENDMENT K BACKGROUND AND RATIONALE

At the time of this submission (October 2010) we have accrued 55 patients to this protocol of which 48 patients have undergone transplant (3 were removed either due to personal request [n =1] or disease progression prior to transplant [n = 2]; the other 4 enrollees are awaiting transplant. As per prior communication, clinical outcomes have either met or exceeded predictions. Relative to safety, all of the criteria for proceeding with the trial (i.e. stopping rules; section **6.4.3**) have been met. Among the 48 transplants there has been one graft failure, which was attributed directly to a poor quality graft, as a second collection from the donor engrafted promptly. The level of acute GVHD has been as expected with grade II-IV acute GVHD being 22% and 33% on the TMS and AC arms, respectively. Grade III-IV acute GVHD has been 11% and 17% on the two arms respectively. Early (< Day +100) treatment-related mortality has been 5% for the entire patient population; the one-year treatment-related mortality has been 17% with no statistical difference in the two arms. Overall survival at one year is greater than 60% on both arms.

As we had hypothesized engraftment was not only nearly universal but was prompt with median full donor chimerism being 100% at day+28 on both arms. We continue to collect data relative to the primary and secondary aims of immune reconstitution and acute GVHD. In regard to the latter our acute are similar to the results reported in the available medical literature for both the TMS and AC arms. A unique aspect is that we continue to prospectively collect data in regard to chronic GVHD using the NIH Consensus Conference diagnosis and staging criteria [61-63]. There are few if any trials comparing two different GVHD regimens which are prospectively studying chronic GVHD using these criteria, which have become internationally accepted and validated retrospectively. The incidence of chronic GVHD among the first 20 evaluable patients have been 60% and 70% on the TMS and AC arms respectively. The incidence of "severe" chronic GVHD, as defined by NIH Consensus Conference criteria, has been 20% on both arms.

As previously stated, chronic GVHD has become a significant research focus in ETIB. These initial chronic results are encouraging, the method of evaluation is unique as the NIH Consensus Conference criteria have been utilized, and a number of highly unique correlative studies have been utilized in this evaluation. Although the data are unique, the number of patients that would potentially be evaluable with the current accrual cap is felt to be insufficient to provide clinically relevant information. Specifically it would be of significant interest to the transplantation community to know if there is (or is not) a difference in severe chronic GVHD between the two GVHD prophylaxis regimens being utilized. In light of the excellent clinical results that have been obtained, we have the unique opportunity to prospectively chronic GVHD and as such have made this a primary objective and have increased accrual number to provide adequate patient numbers to study this clinically important component of allogeneic HSCT.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA – RECIPIENT ON STANDARD CARE THERAPY:

2.1.1 The patient is 18 – 74 years of age [14,30,34].

2.1.2 The patient has a potentially suitable 8/8 donor if they are between the ages of 69-74 years of age or a potentially suitable 8/8 or 7/8 unrelated donor(s) in the National Marrow Registry or Other Available Registry if they are between the ages of 18-74.

2.1.3 The patient currently does not meet the protocol's eligibility/enrollment criteria for any reason.

2.1.4 There is a high likelihood that the patient, in the opinion of the PI will meet the protocol's eligibility/enrollment criteria to proceed to transplant after standard therapy is completed.

2.1.5 The patient or legal guardian is able to give informed consent.

2.2 EXCLUSION CRITERIA – RECIPIENT ON STANDARD CARE THERAPY:

2.2.1 HIV infection. There is theoretical concern that the degree of immune suppression associated with the treatment may result in progression of HIV infection.

2.2.2 Pregnant or lactating. Patients of childbearing potential must use an effective method of contraception (section **4.5**). 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.

2.3 INCLUSION CRITERIA - RECIPIENT

Disease	Disease Status
B-Chronic Lymphocytic Leukemia (CLL) Small Lympho(plasma)cytic Lymphoma (B-SLL, B-LPL) [70,71,72]	a) Relapse/progression after fludarabine and at least one other salvage regimen or 2 standard regimens b) If 17p deletion present, one standard regimen is sufficient
Prolymphocytic Leukemia (PLL) T-CLL [73]	a) T-PLL: Treatment failure after Campath-1H and at least one other regimen b) B-PLL: Treatment failure after fludarabine and at least one other salvage regimen
Hodgkin's Lymphoma [74]	a) Primary treatment failure ineligible for autologous HSCT. Relapse/progression after autologous HSCT.
Follicular Lymphoma, Marginal Zone Lymphomas (splenic, nodal, or extranodal/MALT type) [72,75]	a) Chemotherapy-refractory disease b) Relapse after $\geq$ 2 prior regimens c) Relapse/progression after autologous HSCT
Burkitt or Acute Lymphoblastic Lymphomas [76,77]	a) High-risk disease in remission b) Primary refractory disease c) Recurrent disease d) Relapse/progression after autologous HSCT
Diffuse Large B-cell Lymphoma, Follicular Large cell Lymphoma, Peripheral T-cell Lymphoma, mantle cell lymphoma, Anaplastic Large Cell Lymphoma [78-82]	a) Primary refractory disease. b) Relapse/progression after autologous HSCT after autologous HSCT c) Stable disease or better response to last therapy
Cutaneous T-cell Lymphomas (Mycosis Fungoides, Sezary Syndrome) [83]	a) $\geq$ Stage III b) Disease progression $\geq$ 2 prior regimens, including at least one systemic therapy.
Multiple Myeloma [32,84-86]	a) Relapse/progression after autologous HSCT. b) Plasma cell leukemia c) Adverse cytogenetics: del(13q) or 11q translocation.

Acute Myelogenous Leukemia [87,88]	a) In first complete Remission with high-risk cytogenetics. b) Primary induction failure. c) In second or greater complete remission. d) Secondary AML. e) In first complete Remission with hyperleukocytosis at diagnosis
Acute Lymphocytic Leukemia [89,90]	a) First Complete Remission, with high-risk cytogenetics. b) Primary induction failure. c) Second or greater complete remission.
Myelodysplastic Syndrome [88,91]	a) RAEB I or II b) High-risk IPSS c) Secondary MDS
Myeloproliferative disorders (Idiopathic myelofibrosis, polycythemia vera, essential thrombocytosis, chronic myelomonocytic leukemia, agnogenic myeloid metaplasia) [92,93]	a) Agnogenic myeloid metaplasia with adverse-risk features b) Polycythemia vera or essential thrombocythemia in transformation to secondary AML
Chronic Myelogenous Leukemia [94]	a) Chronic phase CML b) Accelerated phase CML Not eligible for myeloablative allogeneic HSCT
NK Cell Neoplasms	a) First CR for patients with high risk natural killer cell neoplasms including myeloid/NK cell precursor acute leukemia, blastic NK-cell lymphoma, aggressive NK-cell leukemia and nasal-type extranodal NK-cell lymphoma in first complete remission. b) Primary induction failure. c) Second or greater CR.
T-cell Neoplasm: Adult T-Cell leukemia/lymphoma, hepatosplenic T-Cell lymphoma, and enteropathy associated T-Cell Lymphoma	a) First CR b) Chemotherapy-refractory disease c) Relapse after greater than or equal to 1 prior regimen

2.3.1 Diagnosis of hematologic malignancy and at least one of the criteria in the "Disease Status" column as specified in the table above.

2.3.2 Recipients with AML in CR1 must have one of the following:

2.3.2.1 Adverse cytogenetics with residual disease detectable by flow cytometry, cytogenetic analysis, FISH, or PCR. Adverse cytogenetics in AML are defined as complex karyotype (≥ 3 abnormalities); inv(3) or t(3;3); t(6;9); t(6;11); monosomy 7; trisomy 8, alone or with an abnormality other than t(8;21), t(9;11), inv(16) or t(16;16); or t(11;19)(q23;p13.1) [95].

2.3.2.2 Primary induction failure, defined as failure to achieve CR with primary induction chemotherapy [96].

2.3.2.3 Secondary AML, defined as AML related to antecedent MDS, MPD, or cytotoxic chemotherapy.

2.3.2.4 Hyperleukocytosis, WBC ≥ 100,000, at diagnosis.

2.3.2.5 Mutations in the FMS-like tyrosine kinase 3 (FLT3) gene (FLT3-LM; FLT-ITDs) [125, 126, 127]

2.3.3 Recipients with ALL in CR1 must have one of the following:

2.3.3.1 Adverse cytogenetics or residual disease detectable by flow cytometry, cytogenetic analysis, FISH, or PCR. Adverse cytogenetics in ALL are defined as translocations involving t(4;11), t(1;19), t(8;14), 11q23, t(9;22) or *bcr-abl* rearrangement or complex cytogenetic abnormalities.

2.3.3.2 Primary induction failure, defined as failure to achieve CR with primary induction chemotherapy [97].

2.3.4 Patients with Cutaneous T-cell Lymphomas (e.g. mycosis fungoides, Sezary syndrome) must have:

2.3.4.1 Stage III or greater disease

2.3.4.2 Disease which has progressed on or failed to respond to at least 2 therapies including one systemic therapy.

2.3.5 Recipients with agnogenic myeloid metaplasia must have at least 2 of the following features [98,99]:

2.3.5.1 Hemoglobin < 10 g/dl, or > 10 g/dl with transfusion dependence.

2.3.5.2 WBC < 4,000 or > 30,000/mm^3 or requires cytoreductive therapy to maintain.

2.3.5.3 WBC < 30,000/mm.

2.3.5.4 Abnormal cytogenetics including +8, 12p-.

2.3.6 Recipients with primary or secondary acute leukemia, refractory anemia with excess blasts (RAEB), CML, or other eligible diagnosis in transformation to acute leukemia must have ≤ 5% blasts in bone marrow and no circulating blasts in peripheral blood at study entry. Recipients who do not meet these criteria may be re-evaluated for study

eligibility after receiving standard induction therapy for acute leukemia and determined to be in remission.

2.3.7 For recipients with CLL or PLL, treatment failure for a specified therapy is defined as relapse within 6 months or failure to achieve remission.

2.3.7.1 Recipients who are ineligible for a specified therapy (e.g., due to refractory cytopenias) may be considered for enrollment in this protocol.

2.3.7.2 Recipients who have a 17p/p53 deletion who have never received fludarabine will be eligible. [128, 129, 130]

2.3.7.3 Recipients who have had Richter's transformation who have not received prior chemotherapy for their CLL will be eligible.[131]

2.3.8 For patient with NK cell neoplasms: 1) Patients with high risk natural killer cell neoplasms including myeloid/NK cell precursor acute leukemia, blastic NK-cell lymphoma, aggressive NK-cell leukemia and nasal-type extranodal NK-cell lymphoma in first complete remission. 2) All NK cell neoplasms can be transplanted in: a) Primary induction failure or b) Second or greater complete remission.

2.3.9 For patients with T-Cell Neoplasms including adult T-cell leukemia/lymphoma, heptosplenic T-Cell lymphoma and enteropathy associated T-cell lymphoma.

2.3.9.1 First CR.

2.3.9.2 Chemotherapy-refractory disease.

2.3.9.3 Relapse after greater than or equal to 1 prior regimen.

2.3.10 For patients with non-Hodgkin's lymphoma, they must be determined to have at least stable disease to last therapy [82].

2.3.11 For patients with chronic phase chronic myelogenous leukemia, patient's disease must have evidence of never responded to or progressed after receiving a tyrosine-kinase inhibitor (e.g. imatinib mesylate).

2.3.12 Patients with accelerated phase chronic myelogenous leukemia may have $\leq$ 10% blasts in the peripheral smear or bone marrow at study entry.

2.3.13 Patients 18 - 74years of age [14,30,34].

2.3.14 Patient or legal guardian must be able to give informed consent.

2.3.15 All previous cytotoxic chemotherapy must be completed at least 2 weeks prior to study entry, with the exception of the tyrosine kinase inhibitors Imatinib, Nilotinib and Dasatinib which may be continued through induction therapy. 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.

2.3.16 ECOG performance status equal to 0, 1, or 2, and Karnofsky performance status greater than or equal to 60%.

2.3.17 Life expectancy of at least 3 months.

2.3.18 Patients with acute leukemia or myelodysplastic syndrome or chronic myelomonocytic leukemia must be in a hematologic remission, defined as <5% blasts present in both blood and marrow to be referred for evaluation. Should a patient have >5% blasts or a donor not be available by the time the patient is ready for enrollment, the patient will be referred back to their primary hematologist-oncologist for treatment. Patients with diseases other than acute leukemia including but not limited to Hodgkin's and Non-Hodgkin's lymphoma, CLL/SLL, NKTCL, PTCL, must have stable disease to their most recent regimen received within 8 weeks if chemo/radiotherapy or within 12 weeks after prior autologous stem cell transplantation. Should a patient in either of these scenarios have progressive disease or a donor not be available after enrollment, the patient will be referred back to their primary hematologist-oncologist for treatment. If either of these scenarios are not in the best interest of the patient according to the clinical judgment of the PI, then the patient may receive standard treatment for the malignant disease under the current study. If under either of these settings, it becomes apparent that the patient will not be able to proceed to transplant, then he/she must come off study. Recipient-Subjects receiving a standard therapy will be told about the therapy, associated risks, benefits alternatives of the proposed therapy, and availability of receiving the same treatment elsewhere, outside of a research protocol.

2.3.19 Left ventricular ejection fraction > 45% by either MUGA or 2-D echo, obtained within 28 days of enrollment. Patients who have a prior cumulative anthracycline dose greater than 450 mg/m^2 will also have a cardiology consult to determine if further anthracycline administration is an absolute contraindication in patients who may require induction chemotherapy with EPOCH-F.

2.3.20 DLCO > 50% of the expected value when corrected for Hb, obtained within 28 days of enrollment.

2.3.21 Creatinine $\leq$ 1.5 mg/dl and creatinine clearance $\geq$ 50 ml/min/1.73 m^2.

2.3.22 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. Patients with elevations of serum total bilirubin up to 10 mg/dl and/or ALT or AST up to 10 times the upper limit of normal may be considered for participation if such elevations are thought to be due to liver involvement by malignancy. However, in these latter patients, if these values do not decrease to less than or equal to 2.5 times the upper limit of normal during induction chemotherapy, such patients will not be eligible for the transplant phase of the protocol, and will thus be taken off study.

2.3.23 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 if thought to be due to bone marrow involvement by malignancy.

2.4 EXCLUSION CRITERIA - RECIPIENT

2.4.1 Active infection that is not responding to antimicrobial therapy.

2.4.2 Active CNS involvement by malignancy (patients with known positive CSF cytology or parenchymal lesions visible by CT or MRI).

2.4.3 Progressive disease within 8 weeks of prior therapy or within 12 weeks after prior autologous stem cell transplantation.

2.4.4 Active or recent second malignancies unless they 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).

2.4.5 HIV infection. There is theoretical concern that the degree of immune suppression associated with the treatment may result in progression of HIV infection.

2.4.6 Chronic active hepatitis B. Patients may be hepatitis B core antibody positive. For patients with concomitant positive hepatitis B surface antigen, patient will require a hepatology consultation. The risk/benefit profile of transplant and hepatitis B will be discussed with the patient and eligibility determined by the principal investigator.

2.4.7 Hepatitis C infection. Patient may have a hepatitis C infection. However, each patient will require a hepatology consultation. The risk/benefit profile of transplant and hepatitis C will be discussed with the patient and eligibility determined by the principal investigator and lead associate investigator.

2.4.8 Pregnant or lactating. Patients of childbearing potential must use an effective method of contraception (section 4.5). 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.

2.4.9 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 lead associate investigator).

2.4.10 No available suitably HLA- matched unrelated donor.

2.4.11 Available suitably HLA- matched unrelated donor is unwilling to donate PBSC, and no alternate donor is found.

2.5 INCLUSION CRITERIA – DONOR

2.5.1 General donor inclusion criteria specified in the NMDP Standards (20th Edition). (Appendix D)

2.5.2 The evaluation of donors shall be in accordance with existing NMDP Standard Policies and Procedures (Appendix D).

2.5.3 Volunteer unrelated donor matched at a minimum of seven of eight loci (HLA-A, B, C, DRB1), by high resolution typing (>7/8 allele match) are acceptable donors [14, 26, 28,29].

2.5.3.1 The preferred donor-recipient pair would be matched at all eight loci (8/8 allele match).

2.5.3.2 When an 8/8 allele-matched unrelated donor is not available, a single mismatch at HLA-A, -B, -C, or DRB1 will be acceptable in patients who meet all eligility criteria and are 18-69 years of age.

In the situation where more than one 7/8 match is available donor recipient pairs matched at HLA-DQ will be used.

2.5.4 Ability to give informed consent.

2.5.5 Age 18 years or older.

2.5.6 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.

2.6 EXCLUSION CRITERIA – DONOR

2.6.1 Donor exclusion will be in accordance with existing NMDP Standards (20th Edition). (Appendix D)

2.6.2 In addition to NMDP donor exclusion criteria, for the purposes of this protocol, donors who are unwilling to donate PBSC and only wish to pursue a bone marrow donation will be excluded. An alternate donor will be selected if possible, but in the event that no alternate donor is available, the patient will be removed from the trial.

2.6.3 Current treatment with lithium.

2.6.4 Presence of sickle hemoglobin as demonstrated by appropriate testing such as hemoglobin electrophoresis.

2.7 RESEARCH ELIGIBILITY EVALUATION - RECIPIENT

2.7.1 Typing for HLA-A, -B, -C, -DRB1, and –DQB1.

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

2.7.2.1 Complete medical history and physical examination.

2.7.2.2 Nutritional assessment (initial consult).

2.7.2.3 Dental consultation to assess need for teeth cleaning or removal.

2.7.2.4 Social work consultation.

2.7.2.5 Antibody screen for hepatitis A, B, and C; HIV, HTLV-I/II, CMV, adenovirus, EBV, HSV, Toxoplasma, and syphilis.

2.7.2.6 PPD test (if considered to be in a high-risk group).

2.7.2.7 PT, PTT, and ABO typing.

2.7.2.8 Urinalysis.

2.7.2.9 24-hour urine collection for determination of creatinine clearance.

2.7.2.10 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.

2.7.2.11 Chest radiograph.

2.7.2.12 Electrocardiogram and 2D ECHO or MUGA scan.

2.7.2.13 Pulmonary Function Test including DLCO

2.7.2.14 CT scans of chest, abdomen, and pelvis (neck included if measurable disease is present).

2.7.2.15 CT or MRI of the head.

2.7.2.16 Examination of CSF cytology, cell counts and routine chemistries (i.e. glucose and protein) for patients at risk for CNS involvement.

2.7.2.17 PET scan for recipients with non-Hodgkin's lymphoma or Hodgkin's lymphoma.

2.7.2.18 Skeletal survey (for multiple myeloma patients only).

2.7.2.19 Other specific serum or urine diagnostic studies clinically appropriate for the evaluation of recipient's malignancy (e.g., SPEP and UPEP for multiple myeloma).

2.7.2.20 All biopsy specimens will be reviewed by the Laboratory of Pathology/CCR/NCI, for confirmation of the histologic diagnosis prior to enrollment on study.

2.7.2.21 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.

2.7.2.22 Flow cytometric analysis of peripheral blood for baseline measurement of CD3, CD4, CD8, and CD56 positive lymphocyte populations.

2.7.2.23 Baseline determination of serum IL-7 and IL-15 levels, for correlation with NK and T-cell subpopulations.

2.8 RESEARCH ELIGIBILITY EVALUATION - DONOR

2.8.1 Donor eligibility will be determined by the NMDP-affiliated Donor Center Physician according to the most recent and stringent FDA, NMDP, and AABB regulations.

2.8.2 Exceptions to donor eligibility (e.g. foreign travel, tattoos) which do not automatically exclude the donor will be reviewed by the Principal Investigator. The recipient will be informed and consented to any exceptions which could potentially increase the risk of their transplant.

2.8.3 The following clinical, laboratory and radiologic assessments will be performed by the NMDP donor center. Documentation of donor clearance and consent will be delivered to the transplant center through the NMDP search coordinating unit.

2.8.3.1 Complete medical history and physical examination.

2.8.3.2 Antibody screen for hepatitis B, and C; HIV, HTLV-I/II, CMV, HSV, and syphilis. Hepatitis A, Adenovirus, EBV, and Toxoplasma screens will be performed upon receipt of the PBSC product if additional donor blood is available at the time of collection. The results of these screens do not affect the eligibility of the donor for collection or inclusion on this protocol.

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2.8.3.3 CBC with differential, PT, PTT, and ABO typing.

2.8.3.4 Acute care panel, hepatic panel, and mineral panel.

2.8.3.5 Urinalysis.

2.8.3.6 Urine βHCG in women of childbearing potential.

2.8.3.7 Chest radiograph.

2.8.3.8 Electrocardiogram.

2.8.3.9 PCR test of DNA mini-satellite regions for future determination of chimerism will be performed at NIH

2.8.3.10 Data on donor characteristics will be entered in the C3D database for later assessment

2.9 PATIENT REGISTRATION

2.9.1 Protocol Entry

The patient's entry date on protocol is considered to be the day that consent form has been signed by the recipient and confirmation from the NMDP that the requested donor is available, meets the inclusion criteria, and had provided informed consent relative to stem cell donation. The treatment start date is considered to be the day the recipient begins his/her initial induction cycle (Section 3.3).

2.9.2 Registration

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of the patient (recipient) signing consent. The CRO will provide the randomized treatment assignment to the PI based on random assignments determined by the study statistician. Following amendment F, patients will be stratified for 7/8 or 8/8 HLA allele matches, and will be randomized separately according to this stratification. The patient may not sign consent before the patient has been determined to be eligible and confirmation of donor clearance has been received from the NMDP. The patient will not be registered before she/he has signed consent and confirmation from the NMDP that the donor has signed the NMDP "Statement of Intent to Donate" form at the respective institution at which the donor stem cells will be collected. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at the CRO, the CRO staff will call Pharmacy

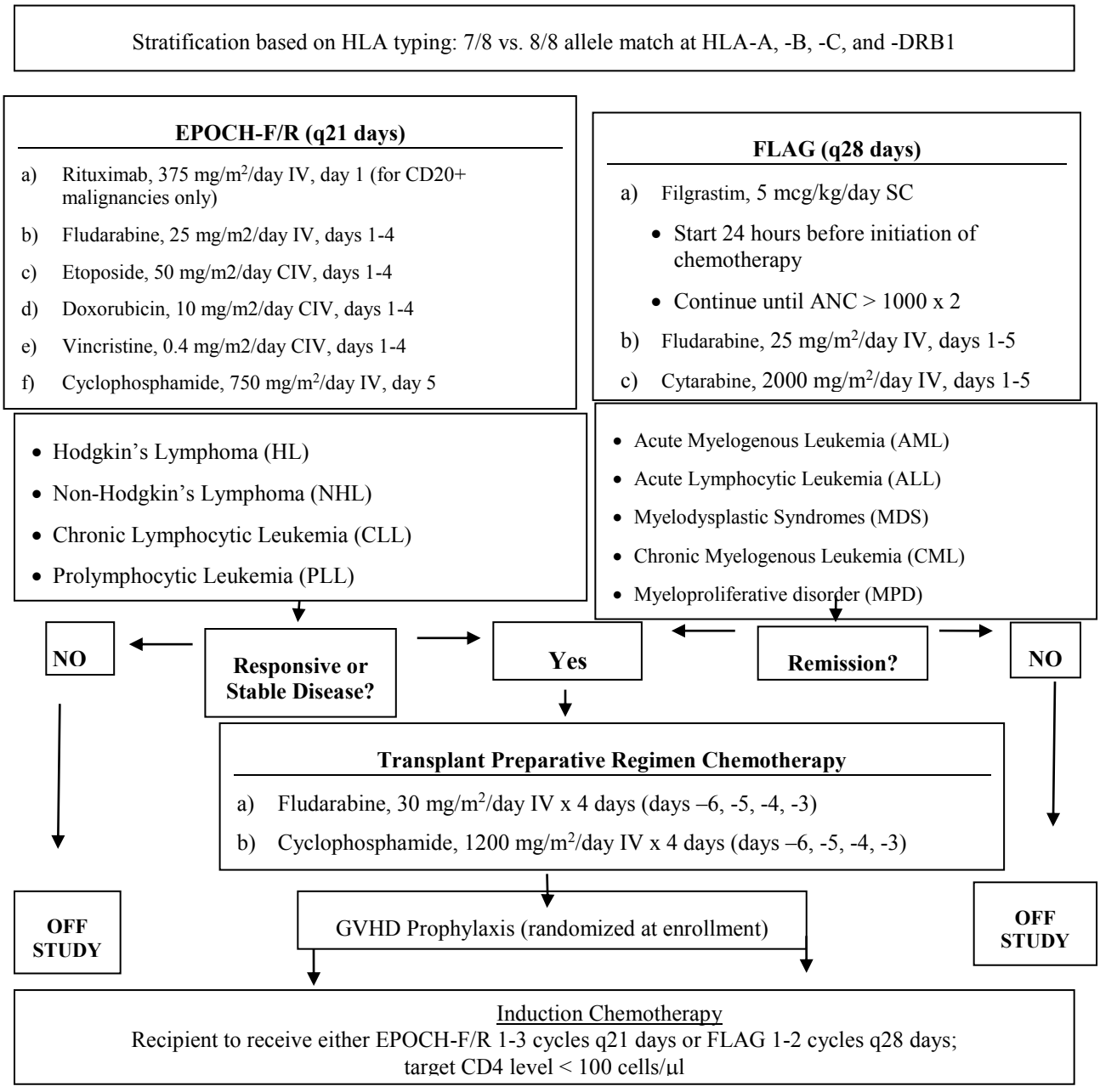
to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents and their randomized treatment assignment. Verification of Registration will be forwarded electronically via e-mail. Please note, it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient's information. A recorder is available during non-working hours.

2.9.3 Off Protocol Therapy and Off-Study Procedure

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

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN



GVHD Prophylaxis (Arm TMS)

a) Tacrolimus 0.02 mg/kg CIV, start day –3. Continue IV or PO. Taper will begin at day +63 if no acute GVHD then at day +119 and discontinue at day +180 as tolerated.
b) Methotrexate 5 mg/m^2 IV, days +1, +3, +6, and +11.

b) c) Sirolimus - initial loading dose of 12 mg p.o. on day -3 pre-transplant and subsequently 4 mg/day. If no acute GVHD then taper at day, +63,

GVHD Prophylaxis (Arm AC)

a) Cyclosporine 2 mg/kg IV Q12h, start day –1. Continue IV or PO. If no acute GVHD at day +100, taper cyclosporine by 10% per week between day 100 and 6 months.

b) Alemtuzumab (Campath®) at 20 mg/day IV over 8 h on days −8 to −4 pre-transplant.

Allogeneic Hematopoietic Stem Cell Transplantation

a) Mobilized PBSCT, day 0 (requested dose $\geq$ 7 x 10^6 CD34+ cells/kg)

b) Filgrastim, 10 mcg/kg/day SC, starting day 0, Continue until ANC > 5000 x 1 day and 1000 x 3 days

Study accrual: number transplanted = 44arm (88 total) for 8/8 and 13/arm (26 total) for 7/8.

Study total = 76 transplanted patients

3.2 Donor Stem Cell Mobilization and Collection (as per NMDP Guidelines)

3.2.1 Donors will receive filgrastim for stem cell mobilization at a dose of 10 to 12 µg/kg/day by subcutaneous injection, according to the NMDP PBSC Protocol (Appendix E). The filgrastim dose will not exceed 1200µg/day. Per NMDP standards, filgrastim will be administered at approximately the same time each day for the first four days. The fifth dose will be given at least one hour prior to apheresis. There will be an additional, sixth dose of filgrastim in cases where a second PBSC is performed on day 6.

3.2.2 Apheresis will start on the fifth day of filgrastim administration. 12-24 liters will be processed daily based on recipient weight (Table 3). The actual volume processed may be adjusted by the NMDP Donor Center or Transplant Center Medical Director based on the pre-collection CD34+ count in order to meet the desired cell dose.

3.2.3 The target cell dose will be greater than or equal to 7 x 10^6 CD34+ cells/kg-recipient weight.

3.2.4 In the event that the donor collection is terminated early for donor-related medical concerns, the donor will be offered the opportunity to consent to a bone marrow donation, and this protocol will allow the infusion of a bone marrow graft. This is considered only in the unlikely circumstance where the recipient has already received conditioning and the product is being collected simultaneously. The recipient will be removed from the study, but will be continued to be managed for all transplant-related care issues and complications.

Table 3. Blood Volume Processed in Relation to Recipient Weight

Recipient Weight (kg)	Volume Processed (L)	Procedure
$\leq$ 35	12	Single 12 liter apheresis.
36 – 45	15	Single 15 liter apheresis.

46 – 55	18	Single 18 liter apheresis or two 12 L aphereses.
56 – 65	22	Single 22 liter apheresis or two 12 liter aphereses.
> 65	24	Single 24 liter apheresis or two 12 liter aphereses.

3.2.5 Cells may be processed, cryopreserved, and stored in liquid nitrogen until the day of transplant upon delivery to the NIH Department of Transfusion Medicine (DTM). In the event that cryopreservation is required, special permission will be obtained from NMDP.

3.2.6 If an ABO incompatibility exists between the donor and patient, the graft will be processed in the cell processing laboratory, according to standard DTM operating procedures.

3.3 THE FOLLOWING ITEMS SHOULD BE OBTAINED WITHIN 48 HOURS BEFORE STARTING INDUCTION CHEMOTHERAPY:

3.3.1 Adequate central venous access is required for all patients undergoing transplantation on this protocol.

3.3.2 CBC with differential.

3.3.3 Acute care panel, hepatic panel, and mineral panel.

3.3.4 Urine βHCG in women of childbearing potential.

3.4 THE FOLLOWING TESTS MAY BE PERFORMED AT ANY TIME PRIOR TO TREATMENT ARM ENROLLMENT

3.4.1 Typing for HLA-A, -B, -C, -DRB1, and –DQB1

3.4.2 Risk of relapse assessment (see Appendix I)

3.4.3 Comorbidity Index assessment (see **Appendix** J)

3.5 INDUCTION CHEMOTHERAPY FOR THE RECIPIENT

3.5.1 A major goal of the induction chemotherapy is to establish severe host immune T cell depletion prior to the allogeneic HSCT. However, there may be individuals who have already experienced severe host T cell depletion with their most recent therapy and additional chemotherapy prior to the transplant conditioning regimen may be placing additional, unnecessary toxicities upon these individuals. As such, patients meeting all of the following criteria will not receive induction chemotherapy and proceed directly to transplant:

3.5.1.1 Fewer than 100 CD4+ cells/ μl and/or within the statistical margin of error of the CD4+ T-lymphocyte depletion goal ≤ 115 CD4 cells/μl of blood at study enrollment, which must be at least 28 days beyond the start of last cycle of therapy prior to enrollment.

3.5.1.2 Demonstration of response that is at least 28 days beyond the start of cycle of therapy prior to enrollment.

3.5.2 Induction Chemotherapy - After enrollment, recipients will receive either EPOCH-F/R or FLAG induction chemotherapy according to their underlying diagnosis, as indicated in the following table [96,97]:

Diagnosis	Induction Chemotherapy Regimen
Acute myelogenous leukemia (AML) Secondary AML Acute lymphocytic leukemia (ALL) Lymphoblastic lymphoma Myelodysplastic syndrome (RAEB) Myeloproliferative disorder (MPD) Chronic myelomonocytic leukemia (CMML) Chronic myelogenous leukemia (CML)	FLAG
Hodgkin's lymphoma Non-Hodgkin's lymphoma (NHL) (except lymphoblastic lymphoma) Chronic lymphocytic leukemia (CLL) Prolymphocytic leukemia (PLL) Multiple myeloma	EPOCH-F/R

3.5.3 EPOCH-Fludarabine/Rituximab Induction Chemotherapy (EPOCH-F/R):

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 PO daily for 5 days	Days 1, 2, 3, 4, 5
Filgrastim	5 μg/kg per day SC	Daily from day 6 until ANC > 1000/μl x 2 following nadir.

3.5.3.1 Only patients with CD20+ B-cell malignancies will receive rituximab as part of the induction chemotherapy regimen, as per Section 2.5.2.20. 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 to a maximum rate of 400 mg/hr. However, if the first infusion is not tolerated well, then the guidelines for the initial infusion should be followed for subsequent administration. Pre-medication with acetaminophen 650 mg PO and diphenhydramine 25 to 50 mg I.V./p.o. 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.

3.5.3.2 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.

3.5.3.3 Etoposide, doxorubicin, and vincristine will be prepared and administered according to NIH Clinical Center Pharmacy standard guidelines.

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

3.5.3.5 Fluconazole should not be taken during the period of EPOCH-F/R administration.

3.5.3.6 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.

3.5.3.7 Filgrastim will be initiated on day 6 at a dose of 5 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.

3.5.3.8 EPOCH-F/R Dose Modification for Cycles 2 and 3:

- Dose adjustments above starting dose level (level 1) consist of a 20% escalation in the daily doses of etoposide, doxorubicin, and cyclophosphamide, relative to the last cycle administered on study.
- Dose adjustments below starting dose level (level 1) consist of a 20% reduction in the cyclophosphamide dose only, relative to the last cycle administered on study.
- Dose adjustments are based on non-hematologic toxicities and the duration of ANC nadir during the previous cycle, using 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.

3.5.3.9 Fludarabine will be reduced by 20% for patients with creatinine clearance less than 70 ml/minute/1.73 m^2.

3.5.3.10 The number of cycles of EPOCH-F/R are specified in section **3.5.5**.

Rules for dose adjustment are summarized in the following table:

• ANC < 500/µl on 1 or fewer measurements • No grade III or IV non-hematologic toxicity	→	Increase 1 dose level above previous cycle.
• ANC < 500/µl on 2 measurements, or transient (≤ 7 days) grade III non-hematologic toxicity • No persistent (> 7days) grade III non-hematologic toxicity • No grade IV non-hematologic toxicity	→	Same dose level as previous cycle.

• ANC < 500/μl on 3 measurements, or persistent grade III non-hematologic toxicity, or transient grade IV non-hematologic toxicity	→	Decrease 1 dose level below previous cycle.
• ANC < 500/μl on 4 or more measurements, at dose level 1 or lower; or • Persistent grade IV non-hematologic toxicity	→	Discontinue EPOCH-F/R; proceed to transplant conditioning regimen.

3.5.4 Fludarabine/Cytarabine/Filgrastim Induction Chemotherapy (FLAG):

Drug	Dose	Days
Fludarabine	25 mg/m^2 per day IV infusion over 30 minutes, Daily for 5 days	Days 1, 2, 3, 4, 5
Cytarabine	2,000 mg/m^2 IV infusion over 4 hours, Daily for 5 days	Days 1, 2, 3, 4, 5
Filgrastim	5 μg/kg per day SC beginning 24 hours PRIOR to initiation of chemotherapy	Daily from day 0 until ANC >1000/μl x 2 following nadir (Section 3.3.4.1)

3.5.4.1 Filgrastim will be initiated within 24 hours prior to the initiation of cytarabine and fludarabine (the day prior to the first day of chemotherapy or Day 0). Filgrastim will be administered at a dose of 5 μg/kg/day will be continued until the ANC is greater than 1000 cells/μl on two consecutive days following ANC nadir.

3.5.4.2 Begin each cytarabine dose 3.5 hours after completion of the preceding fludarabine dose.

3.5.4.3 Corticosteroid ophthalmic drops will be administered 2 drops to each eye every 6 hours, starting prior to first dose of cytarabine and continuing until 24 hours after the last dose of cytarabine has been completed.

3.5.4.4 In the event of signs of CNS toxicity, the cytarabine infusion will be interrupted and the M.D. notified. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine.

3.5.4.5 FLAG Dose Modification for Cycle 2 (individual recipients)

- If the prior cycle of induction chemotherapy is associated with reversible grade 4 non-hematologic toxicity, dose reduction for that patient's subsequent cycles will be performed. Dose reduction will be as follows:
- Fludarabine and cytarabine will be reduced from 5 days to 4 days of administration. The doses of all other medications will remain unchanged.

3.5.4.6 FLAG Dose Reduction (study population):

- If any 2 of 4 consecutive recipients experience the same grade 4 non-hematologic toxicity, dose reduction will be performed for all subsequent cycles for all patients. Dose reduction will be as follows:
- Fludarabine and cytarabine will be reduced from 5 days to 4 days of administration. The doses of all other medications will remain unchanged.

3.5.4.7 Fludarabine will be reduced by 20% for patients with creatinine clearance less than 70 ml/minute/1.73 m2.

3.5.4.8 The number of cycles of FLAG are specified in 3.3.5.

3.5.5 Determination of Number of Cycles of Induction (FLAG or EPOCH-F/R) Chemotherapy:

3.5.5.1 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.

3.5.5.2 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 a CLIA certified laboratory using a 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.

3.5.5.3 If a patient has fewer than 100 CD4 cells/μl and/or within the statistical margin of error of the CD4+ T-lymphocyte depletion goal $\leq$ 115 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.

3.5.5.4 Patients will receive the second cycle of chemotherapy <u>as specified:</u>

- EPOCH-F/R will begin the second cycle of chemotherapy on day 22 after the first cycle was initiated. Patients must have a post-nadir ANC >500/μL and post-nadir platelet count >50,000/μL in order to proceed to next cycle. 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 or platelet recovery, documented infection, or grade III or higher III non-hematologic toxicity resulting from the induction chemotherapy regimen).
- FLAG will begin on day 28 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).

3.5.5.5 Patients who develop excessive neutropenia during chemotherapy will go straight to transplantation

- If a patient receiving EPOCH-F/R 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 ≥ 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.
- If a patient receiving FLAG chemotherapy continues to have neutropenia defined as ANC<500 which continues until ≥ day 28 of treatment, the patient will receive no further induction chemotherapy even if the CD4 count at the time of recovery is ≥ 100 cells/μl.

3.5.5.6 In patients who previously have received a total anthracycline dose > 450mg/m^2 a MUGA or 2-D echo will be repeated after each cycle of EPOCH-FR. If the ejection fraction is less than 45% and/or new wall motion abnormalities have occurred the next planned cycle will be held for 2 weeks and a repeat MUGA or 2-D echo will be performed. If the abnormalities persist the patient will be removed from protocol. If they have resolved the patient may proceed with the next planned course of EPOCH-FR however the Adriamycin will be removed.

3.5.5.7 If the maximum cycles (3 for EPOCH-F/R and 2 for FLAG) of induction chemotherapy has been administered on the study or prior to the study enrollment, then the patients may proceed to the transplant preparative regimen, even if the CD4 count is still ≥ 100 cells/μl.

3.5.5.8 Patients who develop progressive disease or relapse during or after the completion of the induction chemotherapy will not be eligible for the transplant phase of the protocol, and will be removed from the study (Section **3.10**).

3.5.6 Concurrent Therapy for Extramedullary Leukemia or CNS Lymphoma:

3.5.6.1 Concurrent therapy or prophylaxis for testicular leukemia, CNS leukemia, and CNS lymphoma including standard intrathecal chemotherapy and/or radiation therapy will be allowed as clinically indicated after approval by the PI. Such treatment may continue until the planned course is completed. Subjects must be in CNS remission at the time of protocol enrollment.

3.5.7 Concurrent Therapy for bcr-abl (+) CML, AML or ALL

3.5.7.1 Patients receiving tyrosine kinase inhibitors (e.g. imatinib, nilotinib, and dasatinib) may continue through induction therapy. Such treatments must be discontinued one week prior to initiating the conditioning regimen.

There has been an increasing amount of data over the past three years showing that the administration of TKI's with induction and consolidative chemotherapy provides clinical benefit relative to remission. The proposed amendment would allow for the continued use of tyrosine kinase inhibitors though induction therapy. The concomitant use of TKI with conventional chemotherapy is becoming the standard of care for patients with bcr-abl(+) leukemias. The Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) treated 45 patients with newly diagnosed Ph+ ALL and initiated imatinib with consolidative chemotherapy (HAM: mitoxantrone with intermediate-dose cytarabine) consolidation in good early responders (cortico-sensitive and chemo-sensitive ALL) or earlier during the induction course in combination with dexamethasone and vincristine in poor early responders (cortico-resistant and/or chemo-resistant ALL). Imatinib was then continuously administered until stem cell transplantation. Overall, complete remission (CR) and BCR-ABL real-time quantitative polymerase chain reaction (RQ-PCR) negativity rates were 96% and 29%, respectively. All of the 22 CR patients (100%) with a donor actually received allogeneic SCT in first CR. At 18 months, the estimated cumulative incidence of relapse, disease-free survival, and overall survival were 30%, 51%, and 65%, respectively. These 3 end points compared very favorably with results obtained in the pre-imatinib LALA-94 trial. The authors of the paper concluded that their study confirmed the value of the combined approach and encourages prospective trials to define the optimal chemotherapy that has to be combined with imatinib. In addition, it appears that the use of TKI prior to transplant is not associated with increased transplant-related morbidity (TRM) or poorer outcomes [122, 123].

3.5.8 Interim Evaluation During Induction Chemotherapy

3.5.8.1 The following studies will be obtained in all EPOCH-F/R or FLAG recipients during each cycle of induction chemotherapy on a biweekly basis, at least 3 days apart:

3.5.8.1.1 Complete blood count with differential

3.5.8.1.2 Acute care, mineral and liver panel & LDH.

3.5.8.2 The following studies will be obtained in recipients with lymphoma, CLL, PLL, or multiple myeloma at the end of each cycle of EPOCH-F/R induction chemotherapy:

3.5.8.2.1 Routine chemistry hematology panels and 24 hour Creatinine Clearance (if clinically indicated).

3.5.8.2.2 Urine or serum βHCG in women of childbearing potential

3.5.8.2.3 For recipients with lymphoma: computed tomography scans of chest/abdomen/pelvis (and neck, if clinically indicated).

3.5.8.2.4 For recipients with multiple myeloma: serum protein electrophoresis; serum Ig level (free light chains); 24h collection of urine for urine protein electrophoresis, immunofixation, albumin, and creatinine clearance; β_2-microglobulin; immunofixation if M protein is undetectable; CRP

3.5.8.3 The following studies will be obtained in recipients with acute leukemia, lymphoblastic lymphoma, MDS, MPD, CMML, or CML at the end of each cycle of FLAG induction chemotherapy:

3.5.8.3.1 Routine chemistry hematology panels and 24 hour Creatinine Clearance (if clinically indicated).

3.5.8.3.2 Urine or serum βHCG in women of childbearing potential

3.5.8.3.3 For recipients with lymphoblastic lymphoma: computed tomography scans of chest/abdomen/pelvis (and neck, if clinically indicated).

3.5.8.3.4 Bone marrow aspirate and biopsy, with flow cytometry, cytogenetics, FISH and/or PCR as clinically appropriate to monitor disease status.

3.6 TRANSPLANT CONDITIONING REGIMEN

3.6.1 The following studies will be obtained in the recipient within 28 days before the initiation of the conditioning regimen:

3.6.1.1 Bone marrow aspiration and biopsy; flow cytometry, cytogenetics, and molecular studies as clinically appropriate

3.6.1.2 For recipients with lymphoma, CLL, or PLL: computed tomography scans of chest/abdomen/pelvis (and neck, if clinically indicated); FDG-PET scan, if evaluable disease was present on baseline PET.

3.6.1.3 For recipients with multiple myeloma: serum protein electrophoresis; serum Ig level; 24h collection of urine for urine protein electrophoresis; B2-microglobulin; immunofixation if M protein is undetectable; bone marrow biopsy; skeletal survey.

3.6.1.4 Pulmonary function testing including DLCO

3.6.1.5 2-D echo or MUGA scan

3.6.2 The following studies will be obtained in the recipient within 48 hours of the initiation of the transplantation conditioning regimen:

3.6.2.1 CBC with differential.

3.6.2.2 PT, PTT.

3.6.2.3 Acute care panel, hepatic panel, and mineral panel.

3.6.2.4 Urinalysis and 24 hour Creatinine Clearance.

3.6.2.5 Urine or serum □HCG in women of childbearing potential.

6.2.1.6 PCR test of DNA mini-satellite regions for future determination of chimerism

3.6.3 On day 22 after the start of the last cycle of EPOCH-F/R or day 28 after the start of the last cycle of FLAG chemotherapy, patients will be eligible to start the transplant conditioning regimen and should meet the following criteria (performed after completion of induction therapy) Patients who already met all these criteria at the study enrollment may be considered for proceeding with transplant conditioning per PI decision and in consultation with relevant sub-specialist services when pertinent:

3.6.3.1 ECOG performance status equal to 0, 1, or 2, and Karnofsky performance status greater than or equal to 60%.

3.6.3.2 Left ventricular ejection fraction > 45% by either MUGA or 2-D echo.

3.6.3.3 DLCO > 50% of the expected value when corrected for Hb.

3.6.3.4 Creatinine < 1.5 mg/dl and creatinine clearance > 50 ml/min/1.73 m^2.

3.6.3.5 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.

3.6.3.6 No evidence of active infection that is not responding to antimicrobial therapy.

3.6.3.7 Verification of donor eligibility received from NMDP.

3.6.3.8 Verification of disease status:

a) Recipients with primary or secondary acute leukemia, myelodysplastic syndrome, blastic phase CML, myeloproliferative disorders, or other eligible diagnoses in transformation to acute leukemia must have < 5% blasts in bone marrow and no circulating blasts after induction therapy.

b) Recipients with chronic or accelerated phase CML must have ≤ 10% blasts in the peripheral smear or bone marrow after induction therapy.

c) For patients with non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, CLL, PLL, or other lymphoid malignancy must be determined to have at least stable disease to induction therapy.

3.6.4 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.

3.6.5 Fludarabine/Cyclophosphamide/Mesna:

Drug	Dose	Days

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
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

3.6.5.1 Mesna infusion should start concurrently with cyclophosphamide on day –6.

3.6.5.2 Fludarabine will be reduced by 20% for patients with creatinine clearance less than 70 ml/min/1.73 m^2.

3.6.6 Hydration during the conditioning regimen:

3.6.6.1 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 recipients 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.

3.6.6.2 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.

3.6.6.3 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.

3.6.6.4 During hydration, 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.

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

3.7 ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

3.7.1 On day 0, the recipient will receive PBSC. If the PBSC product is cryopreserved, it will be administered intravenously immediately after thawing. Infused CD3 cell dose per kg recipient weight will be recorded

3.7.2 Diphenhydramine and/or meperidine are preferred for the treatment of acute DMSO toxicities (e.g. chills, myalgias); steroids are not permitted.

3.8 GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS

3.8.1 GVHD Prophylaxis Arm Assignment – At the time of study enrollment, the CRO will inform the investigators of the patient's assignment to their respective GVHD prophylaxis arm. Patients will be randomized to receive one of two specific GVHD prophlaxis regimens at the time of enrollment: Tacrolimus + Methotrexate + Sirolimus (TMS) versus Alemtuzumab + Cyclosporine (AC).

3.8.1.1 Tacrolimus + Methotrexate + Sirolimus (TMS Arm) [36]:

a) Tacrolimus will be initiated on day -3 before the transplant. Tacrolimus will be administered continuous intravenous infusion at 0.02 mg/kg/day. Subsequently doses will be adjusted according to trough levels monitored at least biweekly and/or upon symptoms or alteration in renal function. The target serum level for tacrolimus will be 5 to 10 ng/mL.

b) When the recipient is able to take oral medications (typically 10 to 21 days after transplantation), tacrolimus 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.

c) This dose of tacrolimus will continue until day +63 (+/- 2 days), and then reduced by one-third as long as the severity of GVHD is less than grade 2 and patient is not requiring systemic steroids. Tacrolimus will be subsequently reduced by one third on day +119 (+/- 2 days). Tacrolimus will then be completely discontinued by day +180 (i.e. 6 months) discontinued if there are no signs of GVHD.

d) 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
< 2.0 mg/dL	<5.0 mg/dL	100%
2.0 – 3.0 mg/dL	----	50%
> 3.0 mg/dL	> 5.0 mg/dL	Hold Dose

e) Methotrexate may also be held or dose adjusted at the discretion of attending physician or P.I. for severe mucositis or third space fluid collection (e.g. pleural effusion, ascites, or edema. In addition, leucovorin (folinic acid) rescue may be considered under the direction of the attending physician or PI. The recommended dose regimen of leucovorin is 10mg IV or PO every 6 hours starting 24 hours after methotrexate administration and continued until undetectable methotrexate levels or upon the discretion of the attending physician or P.I.. Leucovorin should be held 12 hours prior to the any subsequent methotrexate dose and not resumed (if indicated) for 24 hours after a methotrexate dose.

f) Sirolimus will be initiated on day –3 before the transplant procedure. Sirolimus will be administered by mouth as sirolimus tablets at an initial "loading dose" of 12 mg, p.o., on day –3 of transplantation.

g) On day –3 pre-transplant and subsequently, sirolimus dosing will be 4 mg, p.o., each day. Sirolimus dose should be adjusted to maintain therapeutic concentrations in the range of 3 to 12 ng/ml, with sirolimus trough levels drawn on a Monday, Wednesday, Friday schedule (if inpatient) or a Monday, Thursday schedule (if outpatient).

h) This dose of sirolimus will continue until day +63 (+/- 2 days), and then reduced by one-third as long as the severity of GVHD is less than grade 2 and patient is not requiring systemic steroids. Sirolimus will be subsequently reduced by one third on day +119 (+/- 2 days). Sirolimus will then be completely discontinued by day +180 (i.e. 6 months) if there are no signs of GVHD.

i) Because voriconazole greatly reduces sirolimus clearance via cytochrome p450 inhibition, the use of voriconazole should be avoided in recipients of sirolimus. However, if voriconazole is deemed necessary while the patient is receiving sirolimus, the dose of sirolimus will be reduced by 90% [98].

j) Fluconazole has less of an effect on sirolimus clearance, and therefore will be used for fungal prophylaxis in all patients.

k) For sirolimus levels, 2.5 cc of blood will be collected into EDTA tubes and analyzed at the NIH Clinical Center.

3.8.1.2 Alemtuzumab and Cyclosporine (AC Arm) [31]:

3.8.1.2.1 Alemtuzumab (CAMPATH-1H, Campath®) at 20 mg/day by intravenous (IV) infusion over 8 h on days −8 to −4 (total alemtuzumab = 100 mg).

3.8.1.2.1.1 Patients are to be pre-medicated with diphenhydramine 50 mg p.o. and acetaminophen 650 mg p.o. 30 minutes prior to each alemtuzumab infusion.

3.8.1.2.1.2 In light of the relatively high risk of infusion-related toxicities, the initial infusion rate should be 0.5 mg/hour, which may increased every 15 minutes as tolerated by increments of 0.5 mg/hour until the target rate of 2.5 mg/hour is achieved.

3.8.1.2.1.3 In cases where severe infusion-related events occur, the rate should be slowed or discontinued and patients should receive hydrocortisone 200 mg intravenously after approval by treating physician. Once determined by the treating responsible physician that the patient is stable, the infusion can be re-initiated or advanced as per guidelines in 3.6.1.2.1.2.

3.8.1.2.1.4 Other drug substances should not be added or simultaneously infused through the same intravenous line that alemtuzumab is administered.

3.8.1.2.2 Cyclosporine will be initiated on the day –1 before the transplant. Cyclosporine 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 at least biweekly and/or upon symptoms or alteration in renal function. The target range for serum cyclosporine levels will be 150–250 μg/ml.

3.8.1.2.3 When the recipient is able to take oral medications (typically 10 to 21 days 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.

3.8.1.2.4 This dose of cyclosporine 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 by six months if there are no signs of GVHD.

3.9 Evaluation During Transplantation

3.9.1 The following studies will be obtained in the recipient during hospitalization for transplantation:

3.9.1.1 CBC twice daily; differential count daily

3.9.1.2 Acute care and mineral panels twice daily; hepatic panel and LDH daily

3.9.1.3 Type and screen every 4 days

3.9.1.4 GVHD prophylaxis (cyclosporine, tacrolimus, sirolimus) trough level twice weekly, starting day +1 or day +2.

3.9.2 Donor/recipient chimerism studies:

3.9.2.1 Baseline determination in donor and recipient (prior to start of respective induction chemotherapy).

3.9.2.2 After transplantation in recipient (+/- 3 days):

a) Peripheral blood lymphoid, myeloid, and total chimerism on day +14, +28, +42, +60, if lymphoid and myeloid are ≥ 95% then will check again at day +100. It will then be

obtained monthly until day +180, and every three months thereafter until day +365(+/- 3 days at all timepoint intervals). .

b) Bone marrow chimerism on days +28, +100, and 12 months. Lymphoid & myeloid subset chimerism will also be measured at these time-points if donor chimerism was < 95% on the previous study.

3.9.2.3 Chimerism may be measured at other time-points if clinically indicated (e.g., to determine effect of manipulating immune suppression in order to increase donor chimerism).

3.9.3 The patient will be evaluated at least twice a week during the first 100 days after transplantation for the development of acute GVHD.

3.10 OFF STUDY CRITERIA

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

3.10.1 The recipient will be removed from protocol for any of the following reasons:

3.10.1.1 Inadequate stem cell collection from the selected donor defined as less than or equal to 2 x 10^6 CD34+ cells/kg. If this rare event should occur, another eligible donor may be enrolled and may undergo filgrastim mobilization and PBSC collection as outlined in Section **0**. If no other eligible donor is available or if there is insufficient time to arrange donor cell collection, then the recipient will be removed from the trial; however, the available cells will be infused and the patient will receive standard post-transplant care.

3.10.1.2 Recipients with acute leukemia (primary or secondary), MDS, MPD, CMML-2, or blastic phase CML must be in remission after FLAG induction chemotherapy (section **3.3.4**), defined as $\leq$ 5% blasts on bone marrow aspirate and the absence of circulating blasts in peripheral blood.

3.10.1.3 Progressive disease during or after completion of EPOCH-F/R induction chemotherapy (section **3.3.3**).

3.10.1.4 Non-hematologic toxicity of $\geq$ grade 3 for the recipient while receiving induction chemotherapy (EPOCH-F/R or FLAG), that does not resolve within 14 days after the next cycle of induction chemotherapy or the transplant conditioning regimen is due to be administered. However, recipients with grade 3 or higher infections may remain on study and continue with protocol treatment, if the infection or infections are responding to antimicrobial therapy and are not felt to pose an excessive risk in the opinion of an infectious diseases consultant.

3.10.1.5 Failure to meet pre-transplant eligibility criteria defined in section**2.7.**

3.10.1.6 The donor or recipient refuses to continue therapy.

3.10.1.7 Lost to Follow-Up

3.10.1.8 Death

3.10.2 In all cases, the reason(s) for withdrawal will be documented.

3.10.3 In addition, a patient may at any time be removed from protocol at the principal investigator's discretion, if the P.I. deems the subject 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 POST-TRANSPLANTATION EVALUATION

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

3.11.2 The recipient will be seen in follow-up at the Clinical Center to evaluate disease status and late complications of allogeneic HSCT, at days +28 and +100; and at 6, 9, 12, 18, 24 months and then annually post-transplant thereafter up until the 5 year timepoint unless earlier evaluation is clinically indicated. At these times recipients will undergo the following tests to determine clinical response:

3.11.2.1 Routine chemistry and hematologic panels.

3.11.2.2 Bone marrow aspiration and biopsy with flow cytometry, cytogenetics, and molecular studies as clinically appropriate.

3.11.2.3 For recipients 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 (if indicated). For recipients who enter into CR or CRu with negative PET, follow-up evaluation will include a PET scan at 6 and 12 months post transplant. Other time points may be included if clinically indicated.

3.11.2.4 For recipients 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 will be performed as clinically indicated..

3.11.2.5 Pulmonary Function Test (PFT): (exception: D28 evaluation is only if clinically indicated).

3.11.2.6 Patients who have previously received a total anthracycline dose > 450mg/m^2 will also have a MUGA or 2-D echo will at these timepoints.

3.11.3 Any patient who develops chronic GVHD (before or after day 100) and all patients with acute GVHD manifestations at 6, 9, 12, 15, 18, 21 and 24 months will be referred in the Chronic GVHD Clinic as part of their scheduled routine follow-up.

3.11.4 Any patient who experiences post-transplant relapse will be referred to the Natural History of Disease Outcomes post Allogeneic Hematopoietic Stem Cell Transplantation

protocol 11-C-0125. Patients will not be taken off study in this case but can be enrolled on both studies concurrently.

3.11.5 After 5 year follow up all key protocol research endpoints are achieved there would be no protocol scheduled follow up visits and all further follow up would be based solely on clinical decisions by the PI or LAI.

4 SUPPORTIVE CARE

4.1 INFECTION PROPHYLAXIS

(For a full description of infection prophylaxis please refer to the NIH BMT Consortium Supportive Care Guidelines at http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml)

4.1.1 All recipients will receive prophylaxis against *Pneumocystis jiroveci (previously known as Pneumocystis carinii*) pneumonia, beginning with the first cycle of the induction therapy, continuing until transplantation, and resuming at the time of platelet recovery. Pneumocystis prophylaxis will continue until immunosuppression is discontinued and the CD4 count is > 200 for 3 months. Trimethoprim/sulfamethoxazole is the preferred regimen.

4.1.2 All recipients 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 and immunosuppression is discontinued. Fluconazole may be stopped during therapy with an alternative antifungal agent.

4.1.2.1 Although there is evidence that ketoconazole can raise the AUC of imatinib, as well as cyclosporine, there are no specific recommendations within the medical literature that any of these agents require a specific dose modification. However, it is advised to monitor more closely for the known adverse effects of these agents and to adjust or discontinue these agents as appropriate if an adverse event occur.

4.1.3 During the peri-transplant period, recipients will receive prophylactic broad-spectrum antibiotics with ceftazidime from the onset of neutropenia (ANC < 500/µl) or first fever ($T \geq 38.3°C$) until neutropenia resolves. Recipients with allergy to cephalosporin antibiotics will receive an alternative regimen for prophylaxis as recommended by the infectious diseases consultant. This practice is adopted in recognition of the historically high infectious morbidity and mortality associated with allogeneic HSCT from unrelated donors. At other times, antibiotic therapy for neutropenic recipients will be managed in accordance with CDC guidelines. Recipients with neutropenia and fever that persists for longer than 4 days despite broad-spectrum antibiotics will receive empiric antifungal therapy with caspofungin, liposomal amphotericin B, poseconazole, or voriconazole, in accordance with standard practices. Empiric antifungal therapy may be started at other times, if clinically indicated. Voriconazole should not be administered during the time of sirolimus in patients randomized to that arm (section **3.8.1.1**).

4.1.4 All recipients 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 and immunosuppression is discontinued. Valacyclovir will be discontinued while patients are receiving IV acyclovir, valganciclovir, ganciclovir, or foscarnet.

4.1.5 Recipients with positive pre-transplant serology for Cytomegalovirus (CMV) and/or CMV-sero-positive donors will be monitored for CMV reactivation by clinical evaluation and weekly testing with CMV antigenemia or PCR assays as clinically appropriate. CMV reactivation or disease will be treated according to the schema in Appendix A. Weekly monitoring will continue through 6 months post transplant (longer for recipients on continued immunosuppression).

4.1.6 All recipients will undergo monitoring for reactivation of Epstein-Barr virus (EBV) by weekly quantitative PCR assays. Pre-emptive treatment with rituximab and/or donor lymphocyte infusion (DLI) will be considered for recipients with EBV reactivation, in consultation with infectious diseases staff. Monitoring will continue through day +180 post transplantation (longer for recipients deemed at continued high risk by infectious diseases staff).

4.1.7 All patients will receive ursodeoxycholic acid (Ursodiol) for the prevention of hepatic complications after allogeneic stem cell transplantation [99,100]. Ursodiol will start day -6 prior to the transplant and will continue until day +98 post-transplant. Patients weighing less than 90kg will receive 300mg orally twice daily (600mg total dose each day). Those patients weighing more than 90kg will receive 300mg orally each morning and 600mg orally each evening (900mg total dose each day).

4.1.8 Recipients receiving immunosuppression for chronic GVHD will receive penicillin V for prophylaxis against bacterial infections. All recipients will undergo vaccinations as indicated in Appendix A, beginning 6 months after transplantation. Administration of live vaccines will be avoided during the first 2 years after transplantation or in patients with ongoing chronic GVHD or immunosuppression.

4.2 Management of Engraftment Syndrome

Engraftment syndrome may occur at the time of neutrophil recovery. Its clinical manifestations include noninfectious fever, rash, and vascular leak causing non-cardiogenic pulmonary edema, weight gain, and renal insufficiency [101]. Diagnostic criteria and a treatment schema for engraftment syndrome are included in Appendix B.

4.3 Treatment Of Mixed Chimerism Or Persistent/Progressive Disease Post-Transplant

4.3.1 Donor Lymphocyte Infusion

4.3.1.1 Patients with persistent or progressive malignancy post-HSCT, or mixed chimerism that does not improve after tapering or discontinuing immune suppression may be eligible to receive donor lymphocytes (donor lymphocyte infusion, or “DLI”) if they can be obtained from their respective donor.

4.3.1.2 Additional lymphocytes will be collected by apheresis from the patient's original stem cell donor, either in steady state (i.e. no donor therapy) or after filgrastim administration.

4.3.1.3 DLI may be administered alone or after chemotherapy. DLI may be sequentially administered for mixed chimerism or disease progression Recipients with mixed chimerism or disease progression may receive DLI consisting of unmanipulated donor T cells. Alternatively, in cases where additional donor stem cells are desired, the donor product may be administered without lymphocyte purification.

4.3.1.4 Recipients with mixed chimerism at day +28 will undergo repeat chimerism assessment at day +42 (+/- 3 days) and every two weeks thereafter as deemed necessary. If there is evidence of declining donor chimerism on consecutive determinations or if <50% donor chimerism on whole blood, lymphoid, or myeloid subsets at a single determination, recipients without GVHD will be eligible to receive DLI. DLI may be repeated every 4 weeks with dose escalation as indicated above, until donor chimerism > 95% is achieved or until GVHD develops. Cyclosporine, tacrolimus, and sirolimus will be maintained at planned specific doses through day +100 for recipients with mixed chimerism while DLI are administered. Recipients with mixed chimerism beyond day +100 may undergo withdrawal of respective immunosuppressive agents (i.e. cyclosporine, tacrolimus, and/or sirolimus) over 28 days, followed by DLI administration at escalating doses as outlined.

4.3.1.5 Recipients with disease progression and without GVHD will be eligible to receive DLI. DLI may be repeated every 4 weeks with dose escalation as indicated above, until donor chimerism > 95% is achieved or until GVHD develops. For disease progression occurring after day +42 and before day +100, cyclosporine will be withdrawn over 28 days prior to administration of the first DLI.

4.3.2 Other Post-Transplant Therapy - 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.

4.3.3 For patients with bcr-abl (+) CML, AML or ALL who were previously receiving tyrosine kinase inhibitors (e.g. imatinib, nilotinib and dasatinib) prior to transplant may resume this therapy at Day 28.

As part of a prospective study, Carpenter and colleagues at the Fred Hutchinson Cancer research Center in Seattle administered imatinib from the time of engraftment until 365 days after allogeneic stem cell transplant in 22 patients, 15 with Ph+ acute lymphoblastic leukemia = 15; chronic myelogenous leukemia = 7). Before day 90, adults (n = 19) tolerated a median average daily imatinib dose of 400 mg/d (range, 200-500 mg/d), and children (n = 3) tolerated 265 mg/m2/d (range, 200-290 mg/m2/ d). The most common adverse events related to imatinib administration were grade 1-3 nausea, emesis, and serum transaminase elevations. The

conclusion of this trial was that imatinib could be safely administered early after myeloablative allogeneic HCT at a dose intensity comparable to that used in primary therapy [124].

4.4 Treatment of Graft-Versus-Host Disease

4.4.1 In recipients with suspected GVHD, standard clinical criteria and biopsy findings (when clinically indicated) will be used to establish the diagnosis. Acute GVHD will be assessed according to 1994 Consensus Conference on Acute GVHD Grading criteria [106]. See Appendix C for details concerning the grading and management of acute GVHD. Chronic GVHD will be assessed according to 2005 Chronic GVHD Consensus Project [61]. See Appendix Ffor details concerning the assessment of chronic GVHD.

4.4.2 Recipients 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.

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

4.4.4 Recipients who fail to respond satisfactorily to corticosteroids will be considered for second-line immunosuppressive therapy, e.g., tacrolimus, mycophenolic acid, monoclonal antibodies, or experimental GVHD protocols, if they are available.

4.5 Menses Suppression and Contraception

(For a full description of menses suppression and contraception please refer to the NIH BMT Consortium Supportive Care Guidelines at:
http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml)

4.5.1 Pre-menopausal women who have not undergone hysterectomy will be placed on a monophasic oral contraceptive to provide both menses suppression and contraception. This therapy will begin with the first cycle of induction therapy and continue until platelet recovery after transplantation. (>50,000/mm^3 without transfusion). Start treatment prior to the onset of thrombocytopenia usually when withdrawal bleeding begins. Once thrombocytopenia occurs therapy is less likely to be effective. As general recommendations:

- Prescribe Lo-Ovral (300mcg norgestrol and 30 mcg ethinyl estradiol)
- 1 tablet daily. Instruct patient not to take placebo tablets.
- If the patient is on another oral contraceptive, switch her to Lo-Ovral

If Lo-Ovral is started when the patient is bleeding and bleeding persists for more than 2-3 days, increase the dose to 1 tablet twice daily. If bleeding persists for more than an additional 2-3 days (total 5 to 6 days after starting hormones), consult the NIH Gynecology Consult Service.

4.5.2 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.

4.6 Blood Product Support

4.6.1 Recipients will receive packed red blood cells and platelets as needed to maintain Hb > 8.0 gm/dl, and platelets > 10,000/mm^3 (or higher, if clinically indicated). All blood products except for the stem cell product and DLI will be irradiated.

4.6.2 Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused leukocytes and decrease the risk of CMV infection [107].

4.7 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.

4.8 ANTI-EMETICS

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

4.9 HEMATOPOIETIC GROWTH FACTOR SUPPORT

All patients will start filgrastim 10μg/kg/day on day 0 starting after the stem cell infusion. The dose will be given subcutaneously (SC). Filgrastim will be continued until the absolute neutrophil count (ANC) is ≥ 5000/μl for at least one day and ≥ 1000/μl for three consecutive days. Filgrastim may be resumed if ANC drops below 1000/μl.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH

5.1.1 Immunological Studies

5.1.1.1 T-Cell Receptor Vβ Repertoire Analysis by CDR3 Spectratyping ((Hakim lab).

5.1.1.1.1 40 ml of blood will be drawn from the donor and placed into yellow top ACD tubes at a time before administration of G-CSF for stem cell mobilization. This sample permits calculation of spectratype complexity index [53].

5.1.1.1.2 50 ml of blood will be drawn into yellow top ACD tubes at 1, 3, 6, and 12 months post-transplant from the recipient (Hakim Lab).

5.1.1.1.3 These samples will be ficolled and magnetic cell sorting will be used to separate the CD4+ and CD8+ T cell subsets. RNA will be isolated and RT-PCR followed by Vβ spectratype analysis will be performed.

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

5.1.2.1 Baseline evaluation of donor lymphocytes: aliquot of donor apheresis product containing ~50 x 106 mononuclear cells. Performed in Gress/Hakim lab (10/CRC 3-3288; 301-402-3627).

5.1.2.2 For all subjects, in vitro studies of immune characterization will focus on separation of distinct cell subsets by multi-parameter FACS analysis

Specifically, peripheral blood mononuclear cells (PBMC) will be analyzed by flow cytometry for expression of markers indicative of hematopoietic lineage, immune functional subsets, and activation state.

Cell subsets may be analyzed for T cell receptor rearrangement circles (TREC). Cells may be activated in vitro with a number of different stimuli including specific antigens and mitogens which are known to activate distinct pathways of T lymphocyte function. Assays may include T cell proliferation, cytokine production and gene expression, cytotoxic T lymphocyte generation, and antigen presenting cell function.

The specific assays to be used in the on-going data analyses are subject to be modified, deleted or replaced as technology and knowledge in the field evolve during the course of the study without constituting a change in research aims. These assays apply also to those samples received from outside institutions. If a significant departure from this "Immune Characterization" is contemplated based on accumulated data, then the protocol and consent will be amended accordingly to cover the new line of investigation and its potential risks to subjects.

5.1.2.3 60 ml heparinized whole blood for FACS to Gress/Hakim lab (10/CRC 3-3288; 301-402-3627). Baseline before start of respective (EPOCH-F/R or FLAG) induction chemotherapy; days +7, +14, +28, +60, +100; 6 months, 9 months, 12 months, 18 months, 24 months, annually thereafter, and at the onset of either acute and/or chronic GVHD.

5.1.2.4 20 ml sodium heparinized whole blood (two 10 ml green top tube) for FACS to Gress/Hakim lab (10/CRC 3-3288). At end of each cycle of the respective induction regimen; day 0 following administration of transplant preparative regimen.

5.1.2.5 Recipient IL-7 and IL-15 levels, for correlation with NK and T-cell subpopulations (above): 10 ml whole blood (1 10 ml red top tube), to be sent to Frederick for storage. IL-7 levels will be performed in Mackall laboratory (10/CRC 1-3940; 301-402-0215); IL-15 levels will be performed in Gress/ Hakim lab (10/CRC 3-3288; 301-402-3627). Baseline before start of the respective induction chemotherapy; immediately before the initiation of the conditioning regimen; day 0 following administration of transplant conditioning regimen; days +7, +14, +28, +60, +100; 6 months, 9 months, 12 months, 18 months, 24 months, and annually thereafter. Note: IL-7 levels will not include day +60 or time points after day 100.

5.1.2.6 Lymphocyte subpopulations, IL-7, and IL-15 levels may be obtained at other time points of interest (e.g., development of GVHD, attainment of complete remission, progression of disease, etc…).

5.1.3 Correlation of monocyte cytokine expression patterns with GVHD in recipients (Fowler Laboratory, 10/CRC 3-3224; 301-594-4536):

5.1.3.1 40 ml of blood will be drawn in heparinized tubes on day +14 post-SCT. 20 ml of blood will be drawn in heparinized tubes; days +28, +60,+100 Samples will be sent to the Hakim lab. 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.

5.1.3.2 50 ml of blood will be drawn into green top heparinized tubes on day 7 post-transplant. Samples will be sent to the Hakim Lab. 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.

5.1.4 Tumor acquisition for generation of activated tumor derived lymphocytes (Optional).

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

5.1.4.2 To facilitate this evaluation of tumor reactive T cells, patients will be asked to undergo an optional excisional or core biopsy procedure to obtain tumor tissue (i.e. lymph nodes or masses) or additional bone marrow prior to chemotherapy administration and potentially after transplant, if there is residual tumor.

5.1.4.3 The excisional/core biopsy procedure will be primarily applicable to patients with solid tumor masses (lymphomas, plasmacytomas, etc.). The Surgical Consult Service and/or Interventional Radiology Department of NIH Clinical Center will evaluate each case for their capacity to obtain tumor tissue; only cases that they deem at low risk for complication and can be performed without general anesthesia will be asked to undergo the biopsy procedure.

5.1.4.4 For all patients, an additional 5 ml (heparinized syringe) bone marrow aspirate will be obtained at the times scheduled for bone marrow examination as per protocol.

5.1.4.5 Biopsy or bone marrow specimen samples obtained from the O.R. or Interventional Radiology, or after the bone marrow aspiration procedure, respectively, will be sent to the Hakim Lab. This material will be utilized in subsequent in vitro T cell assays.

5.1.4.6 Patients may be asked to undergo a one-to-two liter single line apheresis procedure for lymphocyte acquisition for comparison with the tumor-derived product.

5.1.5 Analysis of post-transplant antibodies that selectively recognize tumor cell surface antigens (Hakim Laboratory).

5.1.5.1 Post-transplant antibodies that selectively recognize tumor cell surface antigens may be capable of mediating antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) and, thereby, contribute to the allogeneic GVT effect. In addition, these antibodies may serve as tools for the discovery of new targets for human monoclonal antibody therapy. Based on 03-C-0077, the Rader laboratory developed a sensitive flow cytometry assay for detection of post-transplant serum antibodies against B-CLL tumor cell surface antigens. In order to identify these antibodies and subsequently the tumor cell surface antigens they recognize, the Rader laboratory has generated human antibody libraries from post-transplant peripheral blood mononuclear cells (PBMCs) for selection on primary B-CLL tumor cells by phage display. The inclusion of post-transplant serum antibodies and PBMCs from B-CLL patients with (i) HLA-matched unrelated donors and (ii) different GVHD prophylaxis regimens provides an important extension of this ongoing study.

5.1.5.2 Only for accrued B-CLL patients on either GVHD prophylaxis regimen arm, 50 ml heparinized whole blood before start of induction chemotherapy as well as 20 ml heparinized whole blood on days 0, +60, +100; 6 months, 9 months, 12 months, 18 months, 24 months, and annually thereafter to be sent to the Rader laboratory (10/CRC 3-3248) to the attention of Dr. Baskar (301-451-8361).

5.1.6 Analysis of changes in immune cell populations and molecular events in the target tissues of chronic GVHD (Gress Lab 10/CRC 3-3288 301-402-3627; 12C121 301-594-5340).

5.1.6.1 The pathogenesis of chronic GVHD is poorly understood and no studies rigorously evaluated cellular and molecular changes that occur in the target tissues of chronic GVHD. Heterogeneity in patient and treatment related factors complicate the group comparisons. Prospectively designed study with planned sequential sample collection is the ideal format that would give the best chance of success in study of this complex problem.

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

5.1.7 Sample Management and Storage (For more information see section **11.7** for Experimental Transplantation and Immunology Branch Preclinical Service Policy for Sample Handling)

5.1.7.1 All samples will be coded and the key will be available to a restricted number of investigators. However, the nature of the study requires that clinical correlation be feasible as part of hypothesis generation. No change in research subject risk is foreseen from the knowledge acquired from study data. However, if in the judgment of the PI, this should change in the course of the study, NCI IRB will be informed to evaluate the eventual need for modification in subject consent process or for re-contacting subjects.

5.1.7.2 Coded samples will be stored frozen at -20 to -180 C in a single location under the restricted control of the Clinical Core laboratory of ETIB.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data will be prospectively collected and entered in real time into the Cancer Center Clinical Data System database (NCI C3D database) (information at http://ccrtrials.nci.nih.gov). It is expected that clinical data be entered into C3D no later than after 10 business days of the occurrence. The NCI PI and research nurse will have access to these data via web access.

Adverse Events will not be collected or reported to the IRB during the Standard Therapy (EPOCH or FLAG) administered prior to the preparative chemotherapy (conditioning) and transplant phase (if applicable) except grade 5 events.

6.1.1 Adverse Events during the Pre-Transplant conditioning therapy phase (Flu-Cy):

6.1.1.1 Grade 1, 2, and 3 adverse events will not be recorded, with the exception of all Grade 3 events that are unexpected, but are possibly, probably or definitely related to the research

6.1.1.2 All expected and unexpected Grade 4 adverse events possibly, probably or definitely related to the research will be recorded with the exception of:

a) Blood/Marrow abnormalities (will include grade 3 & 4 hemolysis)

6.1.1.3 All grade 5 adverse events will be recorded regardless of attribution.

6.1.2 Adverse Events during the Transplant phase (Day 0 to +180):

6.1.2.1 Grade 1, 2 and 3 adverse events will not be recorded, with the exception of all Grade 3 events that are unexpected, but are possibly, probably or definitely related to the research.

6.1.2.2 All expected and unexpected Grade 4 adverse events possibly, probably or definitely related to the research will be recorded with the exception of:

a) Blood/Marrow abnormalities (will include grade 3 &4 hemolysis and cytopenia related to graft failure)

6.1.2.3 All grade 5 adverse events will be recorded regardless of attribution.

6.1.3 Due to time distance from transplant conditioning and inconsistent patient monitoring once they leave NIH, adverse events grade 1-4 during the follow-up phase (beyond Day +180) will not be recorded.

6.1.3.1 However, all patients will be monitored for GVHD and the dates of onset will be recorded for GVHD, which is the main and only relevant transplant related complication after day +180

6.1.3.2 Blood/Marrow abnormalities due to grade 3 &4 hemolysis and cytopenia related to graft failure will be recorded

6.1.3.3 All grade 5 adverse events will be recorded regardless of attribution.

6.1.4 Adverse Events once patient experiences progressive malignancy

In the event of progressive malignancy only Grade 5 adverse events will be recorded. GVHD will also be recorded.

6.1.5 Data collection procedures

6.1.5.1 After obtaining Informed Consent, a file will be created in the database with standardized forms. The NCI C3D will maintain data for described endpoints in the protocol. The medical record will maintain complete records on each patient including any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records. The NCI C3D will serve as the primary source from which all research analyses will be performed.

6.1.5.2 Each subject's record will be reviewed for verification of eligibility by the research nurse, transplant coordinator, and P.I. or authorized designee prior to consent signing. Documentation of this review will be made on the eligibility checklist and a copy kept in each subject's research record. The research nurse will verify enrollment data entered into the C3D database for each subject within 4 weeks of signing consent. Ongoing data entered into C3D will be verified by the research nurse at completion of induction chemotherapy, after day 28 and day 100 post transplant then bi-annually prior to each continuing review submission. Documentation of data verification will be tracked in the C3D database. Also no data will be collected beyond 5 years except for survival.

6.1.5.3 Data will also be sent to the International Bone Marrow Transplant Registry (IBMTR).

6.1.5.4 Additional baseline and follow-up data may be requested by the NMDP which are not required for this protocol. Recipients will be offered the opportunity to participate in a cross-institute, NIH NMDP Research Data Collection protocol available to all recipients of NMDP hematopoietic stem cell products for which consent is optional. This optional data collection protocol ensures compliance with standards described in the Transplant Center Agreement between the NIH Clinical Center and the NMDP.

All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for a minimum of 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per section **8.2** or until removal from study treatment, whichever comes first**.**

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

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

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

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

6.1.6 Long term follow-up guidelines

Patients may obtain these studies within 2 months of desired date and these may be done in other institutions than NIH with documentation. Please note that these should be performed yearly after transplantation either as part of this protocol or in another setting.

a) Dental evaluations day +365

b) TSH, Ferritin and iron studies on day +180, +365, 24 months, 3, 4, 5 years

c) FSH, LH, estradiol, on day +365 and 24 months (females only)

d) Ophthalmological evaluation on day +365

e) DEXA scan on day +365, then yearly if abnormal or on steroids

f) Echocardiogram on day +100, then only if clinically indicated

g) GYN examination (female patients) day +365

h) Urinalysis/protein screening day +180, +365

i) Pulmonary Function Test on day +100; and at 6, 9, 12, 18, 24 months and then annually post-transplant thereafter up until the 5 year timepoint.

6.2 RESPONSE CRITERIA

6.2.1 Complete Response (CR):

6.2.1.1 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 ($\leq$ 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 $\leq$ 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 [104]. Response criteria may be modified upon publication of revised response criteria [109].

6.2.1.2 Chronic lymphocytic leukemia: complete resolution of all detectable signs and symptoms for at least 2 months, with peripheral blood lymphocytes $\leq$ 4K/μl, neutrophils $\geq$ 1.5K/μl, platelets $\geq$ 100K/μl, hemoglobin > 11g/dl (untransfused), bone marrow lymphocytes < 30% without lymphoid nodules.

6.2.1.3 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*.

6.2.1.4 Other leukemias, myeloproliferative disorders, or myelodysplastic syndrome: hematologic remission is defined as normalization of peripheral blood counts (ANC $\geq$ 1,500/μl and platelets > 100,000/mm^3) without circulating blasts, bone marrow cellularity > 20% with normal maturation, fewer than 5% blasts in bone marrow; and no detectable Auer rods [106]. Extramedullary leukemia may not be present. The absence of specific molecular or cytogenetic markers of disease that were previously present further defines molecular or cytogenetic remission, respectively. In the case of myeloproliferative disorders, there must be absence of bone marrow fibrosis with normal hematologic parameters.

6.2.1.5 Multiple myeloma [111]: absence of urine and serum M-components by immunofixation and electrophoresis; 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.

6.2.2 Complete Response/unconfirmed (CRu):

6.2.2.1 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.

6.2.3 Partial Response (PR):

6.2.3.1 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 [108].

6.2.3.2 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).

6.2.3.3 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.

6.2.3.4 Acute leukemias, MDS or myeloproliferative disorders in transformation: All criteria for complete remission are satisfied (Section **6.2.1**), except that the bone marrow may contain > 5% but < 25% blasts, or $\leq$ 5% blasts are present with Auer rods or abnormal morphology.

6.2.3.5 Multiple myeloma:

a) Very good partial response - 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.

b) Partial response – Reduction by > 50% in serum myeloma protein production maintained for a minimum of six weeks.

6.2.4 Stable Disease (SD):

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

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

6.2.5 Relapsed or Progressive Disease (PD):

6.2.5.1 Non-Hodgkin's lymphoma or Hodgkin's lymphoma: a 50% or greater increase in SPD of all measured lesions compared to the smallest previous measurements, or appearance of any new lesion(s).

6.2.5.2 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 ≥ 50% increase in circulating lymphocytes; Richter's syndrome.

6.2.5.3 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.

6.2.5.4 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.

6.2.5.5 Multiple myeloma (requires 2 of the following): an increase in serum M-protein to ≥ 50% above the lowest level or a rise ≥ 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 ≥ 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.

6.3 TOXICITY CRITERIA

6.3.1 The NCI Common Terminology Criteria for Adverse Events Version 3.0 (CTCAEv3, available via NCI web site (http://ctep.cancer.gov/forms/CTCAEv3.pdf) will be utilized for evaluation of toxicity.

6.3.2 Acute and chronic graft-versus-host disease (GVHD) will be graded according to criteria specified in Appendix C.

6.4 STATISTICAL CONSIDERATIONS

6.4.1 Primary objectives:

6.4.1.1 Determination of CD4+ T cell receptor Vβ repertoire - A primary objective of this study is to conduct a pilot trial to determine the effects of two biologically distinct GVHD prophylaxis regimens (tacrolimus/methotrexate/sirolimus [TMS] and alemtuzumab/ cyclosporine [AC]) on immune reconstitution in patients receiving targeted-immune depletion and reduced-intensity allogeneic HSCT from HLA-matched unrelated donors. As part of a comprehensive assessment of immune reconstitution, the study will primarily focus on differences in CD4+ T cell receptor Vβ repertoire and try to determine in a pilot setting if a) use of either of the two regimens in this trial will have a substantial impact on this parameter at three or 12 months post-transplant, and b) if, again within the context of a pilot study, if there is a difference between the two arms with respect to the magnitude of the change from baseline in this parameter at 3

and 12 months. These will data will be complemented by additional analyses on immune reconstitution which include determination of CD4+ T cell receptor Vβ repertoire at other time points (1, 3, 6, and 12 months), changes in CD8+ T cell receptor Vβ repertoire at 1, 3, 6 and 12 months, the kinetics of CD4+ and CD8+ T-cells and NK cell depletion and recovery, IL-7 and IL-15 cytokine levels, and monocyte production of inflammatory cytokines, as part of the secondary objectives.

6.4.1.1.1 This trial was initially designeded to be a pilot study, with patients stratified according to whether they have 7/8 or 8/8 matches. To meet the primary objective of immune reconstitution, a total of 50 patients with 8/8 matches will undergo transplantation, 25 per arm, and be randomized to receive either an alemtuzumab-based prophylaxis (AC) or triple agent prophylaxis (TMS) based on the order they enrolled onto the study. In addition, 26 patients with 7/8 matches, 13 per arm, will also be randomized to undergo transplantation, and will be evaluated separately. In the situation where a patient is assigned to an arm which is temporarily closed as it is being assessed for toxicities relative to early stopping rules (section **6.4.3**), the patient will be randomized to the arm for which accrual is open. The primary evaluation, for which this number was selected, will be with respect to the change in the CD4+ T cell repertoire diversity, as determined by spectratype complexity index, at 3 and 12 months post-transplant as compared to normal healthy donors. The recipient spectratype complexity index within a Vβ family will be determined as a percentage of the number of peaks in its spectratype histogram compared to the number of peaks in the corresponding donor Vβ family spectratype histogram [49]. Any Vβ family with a complexity index of ≥85% was considered to be fully complex. Histogram peaks will be defined by the Genotyper Genescan software analysis program.

6.4.1.1.2 The study was initially designed to allow for 10 patients per arm, in order to have 91% power to detect a difference between the arms that is equal to 1.5 standard deviations of the intra-arm difference in the single primary parameter, spectratype complexity index at 3 months, with a 0.10 two-sided alpha level test. It will be assumed that the comparison will be made with a two-sample t-test, but if the data in either arm are not normally distributed ($p<0.05$ by a Shapiro-Wilks test), then a Wilcoxon rank sum test will be used. Amendment F will increase the size of the study in order to enroll 20 evaluable patients per arm who have 8/8 matches. With 20 patients per arm who have no mismatches, there will be 86% power to detect a difference between the two arms equal to 1.0 standard deviations of the intra-arm difference of the 3 month and 12 month CD4+ T cell repertoire diversity measures, with a 0.05 two-sided alpha level test. It will be assumed that the comparisons will be made with a two-sample t-test. However, if the data on either arm is not normally distributed ($p<0.05$ by a Shapiro-Wilks test), then a Wilcoxon rank sum test will be used. The 20 evaluable patients (10/arm)

from among 26 transplanted who have 7/8 matches will have results compared between the two arms using the same criteria as in the original protocol design.

6.4.1.1.3 Secondly, the difference in CD4+ T cell repertoire diversity between baseline and 3 months, and baseline and 12 months will be made using a paired t-test if the data are normally distributed. The study originally noted that with 10 patients on a given arm, there is 80% power to detect a 1 SD effect size with a two-sided original design, although there are two arms with this comparison being made within each arm, no formal correction for the two comparisons was required. If the data are not normally distributed, then a Wilcoxon signed rank test would be used. With Amendment F, with 20 evaluable patients per arm from among 25 transplanted, there is 80% power to detect a 0.75 SD effect size for each of the two endpoints within an arm, with a two-sided 0.025 alpha level test for each evaluation. This will allow for an overall 0.05 level test for the two comparisons within each arm, assuming that a Bonferroni adjustment is used. Because of the pilot nature of the original design, although there are two arms with these comparisons being made within each arm, no formal correction for the two comparisons will be required. If the data are not normally distributed, then a Wilcoxon signed rank test will be used. The 20 evaluable patients (10/arm) from among 26 transplanted who have 7/8 matches will have results compared within each arm using the same criteria as in the original protocol design. In order to allow for up to 20% of patients who may die prior to the time of the treatment evaluation point, accrual of up to 25 patients per arm will be permitted for the patients with 8/8 matches and 13/arm for the 7/8 matches in order to yield 20/arm and 10/arm who are evaluable for the two strata.

6.4.2 Determine and monitor incidence, organ severity and overall severity of chronic GVHD - As stated above (section **6.4.1.1.1**) the study was originally intended as a pilot, but it was expanded upon meeting initial safety criteria to 50 patients with 8/8 HLA-matched donors and to study patients with 7/8 HLA-matched donors in a pilot manner. Results from this expanded trial indicate that there is an opportunity to study chronic GVHD in a detailed manner (please refer to section 1.5). Specifically it is a primary arm of this study to determine the incidence of severe chronic using the NIH Consensus Conference diagnosis and staging criteria.

6.4.2.1 Patients will continue to be randomized between these two arms as they have been prior to the amendment. Patients will continue to be stratified according to the degree of matching: 7/8 vs. 8/8 matches. This portion of the trial will be conducted in order to obtain modestly precise estimates of the rates of chronic GVHD in patients treated on either AC or TMS so that we may learn more about the effect of AC vs. TMS on this disease and to permit development of a subsequent, more definitive trial in this patient population. As such, patients enrolled on this trial prior to this amendment will be included in the total patients evaluated with respect to this endpoint.

6.4.2.2 Based on data prior to the initiation of this amendment, is has been estimated that approximately 20% of the patients on each arm will eventually develop severe chronic GVHD. If there were 44 evaluable patients on each arm who survive beyond 100 days post transplant, this would allow a 90% two-sided confidence interval around the observed proportion to have a width of +/-10% from the observed proportion when the expected proportion of patients with severe cGVHD is estimated to be 20%.

6.4.2.3 It is anticipated that 90% of all patients enrolled will be evaluable for evaluation of chronic GVHD in that they survive 100 days post transplant or longer. Thus, in order to enroll 88 total evaluable patients, it is expected that approximately 100 would be needed. If 105 were enrolled and if the true fraction surviving to 100 days was 90%, the probability that 88 or more would survive beyond 100 days is 98.3%; if 100 were enrolled, the probability that 88 or more would survive beyond 100 days is 80%. As such, we will plan to set an accrual ceiling at 105 in order to assure accrual of 88 evaluable patients. Approximately 3-5 patients per month are expected to be able to be randomized to this trial if one or two institutions are participating. Thus, with approximately 50 of the 105 already enrolled, it is anticipated that an additional 1-2 years may be required to complete the enrollment of the 105 randomized patients.

6.4.3 In addition, it will be important to determine the overall safety of both of these regimens in the setting of targeted sequential immune-depleting chemotherapy followed by reduced-intensity allogeneic HSCT from HLA-matched unrelated donors, separately for the patients with 8/8 matches and 7/8 matches. An evaluation of engraftment, severe acute GVHD, early and late treatment-related mortality and overall survival will be performed relative to each arm of this trial, for the 7/8 match and 8/8 match strata separately:

6.4.3.1 Graft Rejection - Early stopping for graft rejection based on CD3 chimerism will also be implemented. Any patient for whom the percentage of CD3 cells is <10% at day 100 will be considered to have rejected the graft. A maximum of 15% of patients with graft rejection is the greatest fraction tolerable.

Patients will continue to be accrued to a specific arm provided that no greater than 2 patients in the 10 within that arm have rejected their graft (and no other stopping rules are implemented). The following table, based on binomial probability calculations, shows the probability of having 2 or more patients rejecting their grafts by 10 patients, for a set of possible underlying true probabilities.

Probability of graft Rejection	Probability of early termination due to graft rejection
.05	.09
.10	.26

.15	.46
.20	.62
.25	.72

Thus, if the true probability of graft rejection is about 15%, there is a 46% probability that 2 or more patients will have this occur within the 10 patients on a specific arm and thus need to end accrual to this arm as soon as this can be determined.

6.4.3.2 Acute GVHD - Acute GVHD will be determined actuarially. Kaplan-Meier curves will be constructed for each arm separately. Acute GVHD will be determined by standard criteria [106]. Stopping rules for grade III or IV acute GVHD will also be implemented. It has been noted that 20% grade III or IV acute GVHD is the upper limit for an acceptable rate, so a stopping rule based on exceeding this rate will be implemented.

Patients will continue to be accrued to a specific arm provided that no greater than 2 patients in the 10 within that arm have developed grade III or IV acute GVHD (and no other stopping rules are implemented). The following table, based on binomial probability calculations, shows the probability of having 2 or more patients develop grade III or IV acute GVHD by 10 patients, for a set of possible underlying true probabilities.

Probability of grade III or IV acute GVHD	Probability of early termination due to grade III or IV acute GVHD
.05	.09
.10	.26
.15	.46
.20	.62
.25	.72

Thus, if the true probability of grade III or IV acute GVHD is about 20%, there is a 62% probability that 2 or more patients will have this occur within the 10 patients on a specific arm and thus need to end accrual to this arm as soon as this can be determined.

6.4.3.3 Early treatment-related mortality - It will be important to continuously monitor the treatment-related mortality, and to have a stopping rule in the event that this is excessive. It has been noted that 20% 100-day treatment-related mortality is the upper limit for an acceptable rate, so a stopping rule based on attaining this rate or exceeding or rate will be implemented, separately for each arm. Treatment-related mortality (TRM) is defined as any death occurring within 28

days after transplantation and any death occurring 28 days or more after transplantation in a patient in continuous remission.

Patients will continue to be accrued to a specific arm provided that no greater than 2 patients in the 10 within that arm have died by day 100 due to treatment-related causes (and no other stopping rules are implemented). The following table, based on binomial probability calculations, shows the probability of having 2 or more patients die from treatment-related causes by 10 patients, for a set of possible underlying true probabilities.

Probability of 100 day TRM	Probability of early termination due to 100 day TRM
.05	.09
.10	.26
.15	.46
.20	.62
.25	.72

Thus, if the true probability of day 100 TRM is about 20%, there is a 62% probability that 2 or more patients will have this occur within the 10 patients on a specific arm and thus need to end accrual to this arm as soon as this can be determined.

6.4.3.4 Late mortality - One year treatment-related mortality and one year overall survival will also be evaluated and used to determine accrual may proceed to a given arm. If greater than approximately 33% of patients are determined to have died due to treatment by one year on a specific arm, then that arm of the trial as originally designed will no longer accrue patients.

6.4.3.5 Late treatment-related mortality - Accrual to the particular arm of the trial will stop if 2 or more patients in the first 6 with that arm have treatment-related mortality within the first year, or this has not been met, if 3 or more in the 10 with that arm have TRM within the first year. The following table, based on binomial probability calculations, shows the probability of having 2 or more patients in the first 6 dying from TRM in the first year, or 3 or more of the first 10 dying from TRM in the first year, for a set of possible underlying true probabilities.

Probability of 1 year treatment-related mortality	Probability of early termination due to 1 year treatment-related mortality
.1	.07
.2	.33
.35	.73

.4	.82
.5	.94

Thus, if the true probability of one year TRM is 35%, there is a 73% probability that this rule will be invoked within the 10 patients on a specific arm and thus need to end accrual to this arm as soon as this can be determined.

6.4.3.6 Overall Survival - Monitoring of overall survival will be performed and if 50% or greater patients within 10 or fewer enrolled on a specific arm are ever determined to not survive until one year for whatever reason, then that arm will no longer accrue patients as it was originally designed.

Accrual to the particular arm of the trial will stop if 3 or more patients in the first 6 within that arm have died from any cause within the first year, or this has not been met, if 5 or more in the 10 in that arm have died from any cause within the first year. The following table, based on binomial probability calculations, shows the probability of having 3 or more patients in the first 6 on a specific arm dying from any cause in the first year, or 5 or more of the 10 in that arm dying from any cause in the first year, for a set of possible underlying true probabilities.

Probability of 1 year mortality	Probability of early termination due to 1 year mortality
.3	.16
.4	.39
.5	.64
.6	.84

6.4.3.7 Although ideally, there would be no additional patients accrued pending determination of whether a specific stopping rule must be invoked for a specific arm, to allow continuity of patients being referred into the trial, up to three additional patients may be permitted to be accrued beyond that needed to evaluate the particular stopping rule.

6.4.3.8 It should be noted for any of the above monitoring plans, that observation of the maximum number of events in 10 patients within a specific arm means that subsequent study of this arm, as specified on this protocol, is inadvisable from a safety perspective, although technically the arm was allowed to continue until the full 10 patient accrual goal within an arm has been met.

6.4.3.9 If one arm of the study is temporarily or permanently closed, then accrual will be permitted to the remaining open arm.

6.4.4 *Secondary objectives* – As secondary endpoints, additional immune reconstitution parameters will be evaluated at multiple time points in an exploratory fashion within the context of a pilot study. Since these will be considered secondary endpoints, no formal adjustment for multiple comparisons will be undertaken, and the comparisons will be reported in the context of a hypothesis generating setting.

6.4.4.1 It is anticipated that approximately 1-2 patients per month may be enrolled onto this study. Thus, to accrue a total of 60 evaluable patients to both arms (up to 25 per arm for the patients with no mismatches and 13 per arm for the arms with 7/8 matches) it is anticipated that up to 3 years will be required. To allow for the possibility of a small number of patients who may be inevaluable (e.g. disease progression prior to transplant), the accrual ceiling will be set at 85 patients or 76 patients who receive a transplant, whichever comes first.

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

7 HUMAN SUBJECT PROTECTIONS

7.1 RATIONALE FOR SUBJECT SELECTION

Patients with high-risk hematologic malignancies will be the subjects for this study. Allogeneic HSCT represents a potentially curative treatment for patients with the disease characteristics selected for inclusion (section **2.1.1**). The expected survival of such patients with conventional chemotherapy is approximately one year or shorter. Eligibility in this protocol is limited to adults (age ≥ 18-74 years).

7.2 PARTICIPATION OF CHILDREN

Children will not be enrolled in this study. Immune reconstitution is different between children and adults, which is the primary endpoint of this study.

7.3 PARTICIPATION OF NIH SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **7.4**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

7.4 RISKS/BENEFITS ANALYSIS

There is a potential benefit to the transplant recipient, as demonstrated by the published literature documenting long-term disease-free survival for patients with high-risk hematologic malignancies following allogeneic HSCT. This carefully defined patient population is a reasonable one in which to explore strategies to improve the results of allogeneic HSCT from unrelated donors, which historically has achieved prolonged remission and survival for some patients but has been associated with significant morbidity and high transplant-related mortality. The strategies chosen in this protocol extend our previous work to develop a platform for consistent engraftment of allogeneic cells through immunoablative induction chemotherapy, host T cell depletion, and reduced-intensity conditioning. Studying this platform in the setting of allogeneic HSCT from unrelated donors is a logical, planned extension of our previous efforts.

The potential risks to the transplant recipient have been carefully considered and are felt to be potentially less than would otherwise occur with myeloablative allogeneic HSCT. The risks graft rejection, acute and chronic GVHD, infection, and relapse will be present, as discussed previously.

7.5 INFORMED CONSENT PROCESS AND DOCUMENTATION

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 at the respective donor center as required by NMDP. A signed informed consent document will be obtained from both the recipient and the donor.

Consent forms: The original signed consent goes to Medical Records; a copy will be placed in research record, as per NIH policy, and a copy will be kept with the patient's chart. A copy of the informed consent documents will also be given to the recipient.

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

The informed consent document contains all elements required for consent. In addition, the Principal Investigatoror their designee will obtain oral consent and will be available to answer all patient questions.

7.5.1 Telephone re-consent procedure

Re-consent on this study may be obtained via telephone according to the following procedure: The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject’s signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject’s records.

The informed consent process will be documented on a progress note by the consenting investigator.

8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

8.1 DEFINITIONS

8.1.1 Adverse Event

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

8.1.2 Suspected adverse reaction

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

8.1.3 Unexpected adverse reaction

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

8.1.4 Serious

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

8.1.5 Serious Adverse Event

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

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.6 Disability

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

8.1.7 Life-threatening adverse drug experience

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

8.1.8 Protocol Deviation (NIH Definition)

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

8.1.9 Non-compliance (NIH Definition)

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

8.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

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

8.1.11 MedWatch Reporting

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

8.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

8.2.1 NCI-IRB and NCI Clinical Director Expedited Reporting of Unanticipated Problems and Deaths

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

- All deaths, except deaths due to progressive disease

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

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

8.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

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

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

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

8.3 Data Safety and Monitoring Plan

8.3.1 Principal Investigator/Research Team

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

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

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

8.3.2 Safety Monitoring Committee (SMC)

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

outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8.4 RECORD KEEPING

8.4.1 All patients must have signed an Informed Consent, and on-study eligibility checklist will be filled out by the Research RN and faxed to the Central Registration Office (CRO) before patient is entered on the study.

8.4.2 Complete records must be maintained on patients; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on the NCI C3D database from which formal analyses are done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flowsheets, specialty forms for pathology, radiation, or surgery; adverse event assessment; and off-study summary sheet, including a final assessment by the treating physician.

8.4.3 Paper CRFs (see **Appendix** K) will be used to aid in data collection on this study.

9 PHARMACEUTICAL INFORMATION

9.1 CYCLOPHOSPHAMIDE (CTX, CYTOXAN, NSC-26271)

9.1.1 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.

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

9.1.3 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.

9.1.4 Route of Administration - The cyclophosphamide used in this regimen will be mixed in 100 ml 0.9% sodium chloride, Inj., and given as an IVPB over 30 minutes in the induction regimen (i.e. EPOCH-F/R; Section **3.5.3**). In the preparative regimen (Section **3.6.5**), it is given over two hours.

9.1.5 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 sub-clinical 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 **3.6.6**. 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.

9.2 MESNA (SODIUM 2-MERCAPTOETHANESULFONATE, MESNUM, MESNEX, NSC-113891)

9.2.1 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.

9.2.2 Preparation – Dilute up to 20 mg Mesna/ml fluid in D5W or 0.9% sodium chloride, Inj. Mesna should be started concurrently with the cyclophosphamide in the preparative regimen (Section 3.4.3). 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).

9.2.3 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% sodium chloride injection, or 24 hours in 0.9% sodium chloride injection.

9.2.4 Administration - To be administered intravenously as continuous infusion.

9.2.5 Toxicities - Nausea, vomiting, diarrhea.

9.3 FILGRASTIM (G-CSF, NEUPOGEN®)

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

9.3.2 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.

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

9.3.4 Administration – Subcutaneous injection is preferred. If clinically indicated, filgrastim may be administered as an intravenous infusion over 15 to 30 minutes.

9.3.5 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.

9.4 CYCLOSPORINE (GENGRAF, SANDIMMUNE, NEORAL)

9.4.1 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.

9.4.2 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 8.4.3). Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.

9.4.3 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.

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

9.4.5 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.

9.5 FLUDARABINE (FLUDARA, BERLEX LABORATORIES)

9.5.1 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.

9.5.2 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.

9.5.3 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.

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

9.5.5 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 (interstitial 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.

9.6 ETOPOSIDE, DOXORUBICIN, AND VINCRISTINE (IN EPOCH)

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

9.6.2 Preparation and Administration - Etoposide, doxorubicin, and vincristine will be combined in a single ('3-in-1') admixture [108]. All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine *PLUS* 40 mL overfill (excess) fluid and a proportional amount of drug to compensate for volume lost in administration set tubing.

Etoposide Dose	Volume of Fluid Containing a Daily Dose	Volume of Overfill (fluid + drug)	Volume To Infuse (over 24 hours)	Administration Rate
< 130 mg	528 mL	40 mL	528 mL	22 mL/hour
≥ 130 mg	1056 mL	40 mL	1056 mL	44 mL/hour

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.

9.6.3 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 [108].

9.6.4 Toxicities:

9.6.4.1 Etoposide: nausea, vomiting, stomatitis, diarrhea, thrombocytopenia, neutropenia, and alopecia. Secondary AML has been associated with

etoposide. Bradycardia and hypotension are sometimes observed with etoposide administration.

9.6.4.2 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.

9.6.4.3 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.

9.7 PREDNISONE

9.7.1 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.

9.7.2 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.

9.7.3 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.

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

9.8 RITUXIMAB (RITUXAN)

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

9.8.2 Preparation – The desired dose will be diluted to a final concentration of 1 to 4 mg/mL in either 0.9% Sodium Chloride or D5W.

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

9.8.4 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. Pre-medication will routinely be administered 30 to 60 minutes prior to the beginning of each rituximab infusion, consisting of acetaminophen 650 mg PO and diphenhydramine 25 to 50 mg i.v./p.o..

9.8.5 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.

9.8.5.1 *Fatal and Severe Infusion Reaction*s: 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.

9.8.5.2 *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.

9.8.5.3 *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.

9.8.5.4 *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 re-administration of RITUXAN to patients with any of these mucocutaneous reactions has not been determined.

9.8.5.5 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).

9.9 CYTARABINE (CYTOSINE ARABINOSIDE, CYTOSAR, ARA-C)

9.9.1 Supply – Cytarabine will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as un-reconstituted lyophilized powder (100 mg, 500 mg, 1 g, and 2 g vials). Cytarabine for injection is available for subcutaneous, intravenous, and intrathecal administration.

9.9.2 Preparation – To prepare high dose (2 gm/m^2) cytarabine intravenous infusions, each vial of drug should be reconstituted with sterile water for injection USP (containing no preservative) with the diluent volumes listed below. Note that the manufacturers recommended diluent for cytarabine is bacteriostatic water for injection containing benzyl alcohol. For preparation of intrathecal injection doses and for high dose intravenous dosing regimens, ONLY a preservative-free diluent should be used. The recommended volumes for reconstitution include the following:

Vial Size	Volume of Diluent	Concentration
100 mg	5 ml	20 mg/ml
500 mg	10 ml	50 mg/ml

1 g	10 ml	100 mg/ml
2 g	20 ml	100 mg/ml

The drug will be further diluted in 500 ml of 5% Dextrose Injection or 0.9% Sodium Chloride Injection prior to administration.

9.9.3 Storage and Stability – Vials should be stored at room temperature (25ºC or 77ºF); excursions permitted to 15-30º C (59-86ºF). [See USP Controlled Room Temperature.] Once reconstituted with sterile water for injection, USP the reconstituted vial should be used within 24 hours. After final dilution with 0.9% Sodium Chloride Injection or 5% Dextrose Injection, the prepared dose should be used within 24 hours if stored at room temperature or 48 hours if refrigerated.

9.9.4 Administration – Begin each cytarabine dose 3.5 hours after completion of the preceding fludarabine dose. Intravenous doses should be infused over at least 2 hours.

9.9.5 Toxicity – Acute dose-limiting toxicity with IV administration consists of severe leukopenia and thrombocytopenia. Nausea and vomiting may be dose limiting at higher doses. Other adverse reactions include diarrhea, immunosuppression, anorexia, stomatitis, oral ulceration, flu-like syndrome, fever, hepatic dysfunction, and alopecia. At high doses, as in this protocol, keratoconjunctivitis, dermatitis, and central nervous system toxicity (e.g., ataxia, somnolence, coma, dysarthria) may occur. Occasionally, the CNS impairment is not fully reversible. Renal impairment will enhance toxicity. In the event that signs of CNS toxicity occur, the cytarabine will be interrupted and the M.D. notified. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine.

9.9.6 Conjunctivitis Prophylaxis: Corticosteroid ophthalmic drops will be administered 2 drops to each eye every 6 hours starting prior to first dose and continuing until 24 hours after the last dose of cytarabine has been completed.

9.10 DIPHENHYDRAMINE

9.10.1 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.

9.10.2 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.

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

9.10.4 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).

9.10.5 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.

9.11 ACETAMINOPHEN

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

9.11.2 Storage – Store at controlled room temperature.

9.11.3 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.

9.11.4 Toxicities – No toxicities are anticipated to result from single doses of acetaminophen administered as pre-medication for rituximab infusions.

9.12 VALACYCLOVIR (VALTREX®)

9.12.1 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.)

9.12.2 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.

9.12.3 Storage and Stability – Oral tablets should be stored at 15º to 25ºC (59º to 77ºF).

9.12.4 Administration – Oral.

9.12.5 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).

9.13 FLUCONAZOLE (DIFLUCAN®)

9.13.1 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).

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

9.13.3 Storage and Stability – Oral tablets and oral suspension should be stored at temperatures below 30ºC (86ºF). Store reconstituted oral suspension between 5º to 30ºC (41º to 86ºF)

and discard unused portion after 2 weeks. Fluconazole for injection in glass bottles should be stored between 5º to 30ºC (41º to 86ºF). Fluconazole for injection in Viaflex® Plus plastic containers should be stored between 5º to 25ºC (41º to 77ºF).

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

9.13.5 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.

9.13.6 Drug Interactions – Fluconazole is a potent inhibitor of the cytochrome P450 3A4 isoenzyme system. Co-administration 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.

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

9.14.1 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.

9.14.2 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. 0.9% sodium chloride, Inj. may be substituted as a diluent but the resulting solutions have reduced stability. Consult with pharmacy for questions regarding diluent, volume, and expiration.

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

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

9.14.5 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 irritation on IV administration are infrequent. Thrombophlebitis has rarely been observed.

9.15 FUROSEMIDE (LASIX)

9.15.1 Supply – Commercially available as 20mg, 40mg, and 80mg tablets or as an injectable solution of 10mg/ml, from multiple manufacturers.

9.15.2 Preparation – Furosemide 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).

9.15.3 Storage and Stability – Store commercially available injectable product at controlled room temperature and protect from light exposure.

9.15.4 Administration – Furosemide injection may be administered by direct IV injection (IV push) over 1 to 2 minutes, and by intravenous infusion at a rate not exceeding 4mg/min.

9.15.5 Toxicities – Common toxicities include hypokalemia, hypomagnesemia, hyperuricemia, hyperglycemia, glycosuria, anorexia, constipation, cramping, diarrhea, blurred vision, dizziness, headache, parathesias, vertigo, muscle spasms, weakness, purpura, photosensitivity, pruritus, urticaria, and rash. Uncommon but serious adverse effects include cytopenias, hemolytic anemia, erythema multiforme, Stevens Johnson syndrome, hypotension, and pancreatitis.

9.16 METHOTREXATE (METHOTREXATE SODIUM, MTX, NSC-740)

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

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

9.16.3 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.

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

9.16.5 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.

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

9.17.1 Supply - For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

9.17.2 Storage and Stability - Oral tablets should be stored 20-25 C° (68-77 F°). Oral solution should be refrigerated (2-8 C° or 36-46 F°).

9.17.3 Administration: Because food, in particular fatty foods, can decrease the absorption of sirolimus, it is suggested the tablets should be administered consistently between meals.

9.17.4 Toxicities: 1) Sirolimus induces immune suppression, which has been associated with opportunistic infection and an increased rate of malignancy, particularly skin cancer. 2) Some individuals may develop hypersensitivity to sirolimus. 3) May cause an increase in cholesterol and triglycerides, which may be associated with pancreatitis. 4) With long-term administration, may result in impaired renal function. 4) Metabolism is via the cytochrome p450 pathway, and as such, co-administration of voriconazole is prohibited with sirolimus can result in prolonged blood levels of sirolimus. As such, if the administration of voriconazole is required the dose of sirolimus will be reduced by 90% and close attention must be given relative to serum sirolimus levels and associated toxicities [98].

9.18 ALEMTUZUMAB (CAMPATH-1H, CAMPATH®)

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

9.18.2 Storage and stability – Vials of Alemtuzumab should be stored at 2-8°C (36-46°F) and protected from sunlight. The vial should not be frozen; if the vial has been frozen it should be discarded. Alemtuzumab contains no antimicrobial preservative. An internal NIH Pharmacy (Pharmaceutical Development Section) conducted study demonstrated 24 hour stability of alemtuzumab when diluted in 0.9% sodium chloride to a concentration range of 6.67 mcg/mL to 120 mcg/mL at room temperature (Goldspiel JT, et.al. Stability of alemtuzumab solutions at room temperature. Am J Health –Syst Pharm, accepted for publication 2012) .. Alemtuzumab solutions prepared in the concentration range described above may be stored at room temperature (15-30°C) for up to 24 hours. Alemtuzumab solutions should be protected from light.

9.18.3 Preparation – The vial should be inspected for visible particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. The vial should not be shaken prior to use. The necessary amount of Alemtuzumab should be withdrawn from the vial into a syringe. The vial contains no preservatives and is intended for single use only; the vial should be discarded with any unused portion after 6 hours. The desired dose of 20 mg is then injected into 250 mL

sterile 0.9% Sodium Chloride USP. The bag should be gently inverted to mix the solution. The syringe is discarded.

9.18.4 Administration - Alemtuzumab should be administered intravenously only. The infusion should be administered over an 8 hour period.. Other drug substances should not be added or simultaneously infused through the same intravenous line.

9.18.5 Toxicities:

9.18.5.1 Infusion-related adverse events are very common (>80%) with Alemtuzumab administration. The most commonly reported infusion-related adverse events on this study included rigors, fever, nausea, vomiting, and hypotension. Other frequently reported infusion-related events include rash, fatigue, urticaria, dyspnea, pruritus, headache, and diarrhea. Serious infusion-related events that have been reported include syncope, pulmonary infiltrates, ARDS, respiratory arrest, cardiac arrhythmias, myocardial infarction and cardiac arrest.

9.18.5.2 The following serious adverse events have been were reported in at least one patient treated on studies where Alemtuzumab was used as a single agent to treat malignant (e.g. chronic lymphocytic leukemia) and non-malignant diseases (e.g. rheumatoid arthritis): allergic and anaphylactoid reactions, ascites, cyanosis, atrial and ventricular arrhythmias, angina pericarditis, abnormal gait, aphasia, coma, seizures, hyperthyroidism, duodenal ulcer, GI hemorrhage, intestinal obstruction, intestinal perforation, pancreatitis, hepatocellular damage, respiratory alkalosis, arthritis or worsening arthritis, arthropathy, osteomyelitis, polymyositis, malignant lymphoma, secondary leukemia, disseminated intravascular coagulation, thrombocythemia agranulocytosis, aplasia, lymphadenopathy, hemolytic anemia, splenic infarction, splenomegaly, cervical dysplasia, bacterial infections, *Herpes zoster* infection, *Pneumocystis carinii* infection, otitis media, asthma, pleural effusion, pleurisy, pulmonary edema, pulmonary fibrosis, acute renal failure, cerebral hemorrhage, and deep vein thrombosis.

9.19 URSODEOXYCHOLIC ACID (URSODIOL, ACTIGALL®)

9.19.1 Supply – Commercially available as 300 mg capsules.

9.19.2 Pharmacology - About 90% of a therapeutic dose of Ursodiol is absorbed in the small bowel after oral administration. After absorption, Ursodiol enters the portal vein and undergoes efficient extraction from portal blood by the liver where it is conjugated with either glycine or taurine and is then secreted into the hepatic bile ducts. Only small quantities of Ursodiol appear in the systemic circulation and very small amounts are excreted into urine. The sites of the drug's therapeutic actions are in the liver, bile, and gut lumen.

9.19.3 Storage and Stability – Oral capsules should be stored at 15°C-30°C (59°F-86°F).

9.19.4 Administration – Oral.

9.19.5 Toxicities – Nausea, vomiting, dyspepsia, metallic taste, abdominal pain, biliary pain, cholecystitis, constipation, stomatitis, flatulence, diarrhea, pruritus, rash, dry skin,

urticaria, headache, fatigue, anxiety, depression, sleep disorders. Less common side effects include sweating, thinning of hair, back pain, arthralgia, myalgia, rhinitis, cough.

9.19.6 Drug Interactions - Bile acid sequestering agents such as cholestyramine and colestipol may interfere with the action of Ursodiol by reducing its absorption. Aluminum-based antacids have been shown to adsorb bile acids in vitro and may be expected to interfere with Ursodiol in the same manner as the bile acid sequestering agents. Estrogens, oral contraceptives, and clofibrate increase hepatic cholesterol secretion, and encourage cholesterol gallstone formation and hence may counteract the effectiveness of Ursodiol.

9.20 TACROLIMUS (FK-506, PROGRAF®)

9.20.1 Supply – Tacrolimus will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Tacrolimus is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL for administration by intravenous infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v.

9.20.2 Preparation – For parenteral doses, tacrolimus injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection before use. Parenteral doses of tacrolimus will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing (see Storage and Stability, section 8.4.3).

9.20.3 Storage and Stability – Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light.

9.20.4 Administration – Tacrolimus will be administered as a continuous intravenous infusion over 24 hours or given orally in divided doses every 12 hours.

9.20.5 Toxicities – Tacrolimus can cause neurotoxicity and nephrotoxicity, particularly when used in high doses. Mild to severe hyperkalemia has been observed following tacrolimus administration. Serum potassium levels should be monitored and potassium-sparing diuretics should not be used during tacrolimus therapy. Neurotoxicity, including tremor, headache, and other changes in motor function,0 mental status, and sensory function have been reported with tacrolimus administration. Tremor and headache have been associated with high whole-blood concentrations of tacrolimus and may respond to dosage adjustment. Seizures have occurred in adult and pediatric patients receiving tacrolimus. Coma and delirium also have been associated with high plasma concentrations of tacrolimus. The most common adverse reactions reported with tacrolimus administration include infection, tremor, hypertension, abnormal renal function, constipation, diarrhea, headache, abdominal pain and insomnia. Less common adverse reactions reported with tacrolimus administration include anorexia, cholangitis, jaundice, diarrhea, dyspepsia, hepatitis, ALT (SGPT) increased, AST (SGOT) increased, abnormal ECG, angina pectoris, arrhythmia, acute kidney failure, acidosis, alkaline phosphatase increased, alkalosis, bicarbonate decreased, bilirubinemia, BUN increased, dehydration, edema, hypercalcemia, , hypocalcemia, hypercholesterolemia, hyperlipemia, hyperphosphatemia, hyperuricemiahypoglycemia, hypomagnesemia, hyponatremia, hypophosphatemia, hypoproteinemia, lactic dehydrogenase increase, peripheral edema, weight gain,

Cushing's syndrome, diabetes mellitus, anemia, allergic reaction, and photosensitivity reaction.

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11 APPENDICES

11.1 APPENDIX A - PROPHYLAXIS AND TREATMENT OF INFECTIOUS COMPLICATIONS IN ALLOGENEIC HSCT RECIPIENTS

(Note: The practice guidelines primarily follow the Guidelines for Infection Management as outlined by the NIH Blood and Marrow Transplant Consortium's Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients (http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml). The practice guidelines included in this Appendix are based upon the best available clinical evidence and may change as additional data become available.)

11.1.1 Pneumocystis jiroveci pneumonia

11.1.1.1 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 QD on three days per week. This will continue until completion of induction chemotherapy.

11.1.1.2 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.

11.1.1.3 Prophylaxis is resumed when the absolute neutrophil count is greater than 1,000/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 3.6.1.1.4). The dose for post-transplant prophylaxis is TMP/SMX 160/800, one tablet PO QD on three days per week, continuing until the recipient's CD4

count is above 200 cells/μl for 3 months and all immunosuppressive agents are discontinued.

11.1.1.4 For patients allergic to sulfa, options include aerosolized pentamidine 300 mg by inhalation Q4 weeks, dapsone 100 mg PO QD, or atovaquone 1,500 mg PO QD. Prior to instituting dapsone therapy, G6PD deficiency must be excluded.

11.1.2 FUNGAL PATHOGENS

11.1.2.1 Prophylaxis – Candida infections:

a) On day 6 of the first cycle of EPOCH-F/R induction chemotherapy, recipients will begin fluconazole 400 mg PO daily. Because of its interaction with vincristine, fluconazole will be discontinued during subsequent cycles of induction chemotherapy on days 1 through 5. Fluconazole will then be restarted on day 6 of each cycle.

b) Recipients receiving FLAG induction chemotherapy will receive fluconazole 400 mg PO daily, beginning with day 1 of the first cycle of FLAG.

c) Recipients will continue fluconazole 400 mg PO daily throughout the transplant conditioning regimen (day –6) until 100 days after transplantation and immunosuppression is discontinued. Intravenous dosing will be substituted when patients are unable to tolerate oral medications. Fluconazole will be discontinued when an alternative antifungal is started.

d) Alternatives to fluconazole include caspofungin, posaconazole or itraconazole. Caspofungin dosing is 70 mg IV loading dose on day 1, then 50 mg IV QD. Posaconazole dosing is 200mg PO q 6-8h. Itraconazole dosing is 200 mg IV Q12 hours for 2 days (loading dose), then 200 mg IV Q24 hours for 12 days, then 200 mg PO Q12 hours to maintain levels (itraconazole plus hydroxy-itraconazole) greater than 1 μg/ml. Triazoles should be avoided in recipients with fluconazole hepatotoxicity. In such cases, consult the infectious diseases service.

11.1.2.2 Prophylaxis – Aspergillus infections:

a) <u>During Neutropenia</u>: Neutropenic recipients who are febrile for more than 5 days despite broad-spectrum antibiotics and have no clinical evidence of invasive fungal disease will receive caspofungin 70 mg IV loading dose, then 50 mg IV Q24 hours. An alternative regimen is 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. Empiric antifungal therapy may be started at other times, if clinically indicated.

b) <u>During Steroid Therapy for GVHD</u>: Recipients with GVHD receiving corticosteroids at a dose equivalent to $\geq$ 1 mg/kg/day for $\geq$ 6 days will receive posaconazole 200mg PO q6-8h. An alternative regimen is voriconazole 6 mg/kg IV Q12 hours x 2 doses, then 3 mg/kg IV Q12 hours. Voriconazole therapy will be converted to oral dosing at 200 mg PO Q12 hours when tolerated. Alternatively, voriconazole may be initiated with oral dosing at 400 mg PO Q12 hours x 2 doses, then 200 mg PO Q12 hours. Antifungal prophylaxis with activity against Aspergillus should be continued until the corticosteroid dose is reduced to $\leq$ 0.6 mg/kg/day. Alternative regimens include:

- Amphotericin B lipid complex (ABLC) 5 mg/kg IV QD
- Caspofungin 70 mg IV loading dose followed by 50 mg IV QD
- Itraconazole 200 mg IV Q12 hours x 2 days (loading dose), then 200 mg IV Q24 hours x 12 days. When tolerated, itraconazole may be converted to oral dosing at 200 mg PO Q12 hours to maintain levels (itraconazole + hydroxy-itraconazole) greater than 1 μg/ml.

> *Notes*:
>
> Empirical antifungal therapy during neutropenia is controversial. Less than 10% 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 [113]. The relevance of these results to patients with neutropenia of short duration who are receiving fluconazole prophylaxis is questionable. In the most recent trial of empirical antifungal therapy, breakthrough fungal infections were more prevalent in high-risk patients with relapsed leukemia and/or allogeneic bone marrow transplant than in other febrile neutropenic patients [114]. If persistent fever during neutropenia is a manifestation of 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 caspofungin or high-dose amphotericin B (at least 5 mg/kg). As our allogeneic HSCT recipients receive cyclosporine, a known nephrotoxic drug,after transplantation, amphotericin B lipid formulations are preferred. Voriconazole is a valid alternative, and in the mentioned study was associated with significantly fewer breakthrough fungal infections than Ambisome [114].

11.1.3 Viral Pathogens

11.1.3.1 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 recipients start induction chemotherapy (EPOCH-F/R) and continue throughout transplantation. Valacylovir will continue until 100 days after transplantation and immunosuppression is discontinued. If a recipient cannot take oral medications, acyclovir 250 mg/m^2 IV q12h will be substituted for valacyclovir.

Notes:

- HSV is an important cause of morbidity after high-dose chemotherapy. Several effective regimens are available. Every recipient 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 (see below). Our recipients 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. Acyclovir (400 mg/d) administered until the end of immunosuppressive therapy has been reported to significantly decrease the incidence of reactivation [115]. 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 [116,117]. We prefer valacyclovir for the convenience of once daily dosing, although it

11.1.3.2 Cytomegalovirus (CMV)

a) Preemptive Therapy: Recipients with positive pre-transplant serologies for CMV, or whose donors have positive serologies, will undergo weekly monitoring of CMV by polymerase chain reaction (PCR). CMV monitoring by PCR will begin at the time of engraftment after transplantation and continue through day +180. Recipients who continue to require systemic immunosuppression after day +180 will continue to have CMV monitoring performed weekly.

b) Non-scheduled CMV monitoring may also be warranted for clinical circumstances such as:

- Fever of unknown origin
- Cytopenias post-transplant
- Pulmonary changes

c) If PCR becomes positive, then recipients will be treated with IV ganciclovir or IV foscarnet according to the following algorithm:

Preferred Agents		Alternatives	
IV GANCICLOVIR	IV FOSCARNET	VALGANCICLOVIR	CIDOFOVIR
Induction: 5 mg/kg IV q12h for 7 days, then maintenance: 5 mg/kg IV QD, 5 days/week	Induction: 60 mg/kg IV q12h for 7 days, then maintenance: 60 mg/kg IV QD	Induction: 900 mg PO q12h for 7 days, then maintenance: 900 mg/24h	Induction: 5 mg/kg IV weekly, then maintenance: 5 mg/kg every other week. Include probenecid and hydration.

All patients receive one week of induction. Maintenance therapy should continue until one negative or two "low positive" PCR results (henceforth "DNAemia").? Patients with persistent or rising DNAemia during or after induction therapy for CMV will continue or resume ganciclovir at induction doses (5 mg/kg IV q12 hours for 7 consecutive days), because an increase in DNAemia does not indicate ganciclovir-resistant CMV in most patients during or after initial induction If possible, the virus will be sent for sequencing to rule out the presence of mutations associated with ganciclovir or foscarnet resistance (this is not often possible, as the virus must be isolated and grown in culture). If after reinduction there is no improvement, the patient will be changed to another appropriate antiviral therapy (e.g. foscarnet). Otherwise, the patient will be switched to maintenance therapy as described in the table above. If renal adjustment is required for ganciclovir or foscarnet, see http://druginfo.cc.nih.gov or contact unit pharmacist.

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.

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 re-induction.

11.1.4 Bacterial Pathogens

11.1.4.1 Hypogammaglobulinemia: Recipients will undergo biweekly monitoring of serum IgG levels beginning prior to transplant and continuing until IgG is > 500 mg/dL for at least 3 consecutive months without supplemental IVIG administration. Recipients with IgG ≤ 400 mg/dL AND a history of repeated sinopulmonary infections (≥ 2 respiratory infections requiring systemic antibiotics over 6 months) will receive IVIG 400-500 mg/kg every 2-4 weeks.

11.1.4.2 Prophylaxis During Chronic GVHD: All recipients receiving treatment for chronic GVHD will receive penicillin V 500 mg PO BID until treatment for chronic GVHD is discontinued. For penicillin-allergic recipients, TMP/SMX 80/400 PO QD can be used. For recipients 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 [119]. Functional hyposplenism has been considered, and some investigators have attempted to monitor it by ultrasound [120]. Current recommendations support the use of antibiotic prophylaxis with penicillin to prevent these infections. TMP/SMX could be substituted, but only when administered daily.

11.1.4.3 Immunizations: All recipients will receive immunizations after transplant, according to the following table:

Type	6 Mo	12 Mo	14 – 18 Mo	24 Mo
Influenza Virus	Lifelong, seasonal, starting before HSCT and resuming after 6 months			
Pneumococcal 7-valent conjugate (0.5 mL, IM)	X	X		
DTaP [a] (0.5 mL IM)		X	X	X
Hib [b] conjugate (0.5 mL IM)		X	X	X
IPV [c] (0.5 mL SQ or IM)		X	X	X
Hepatitis A (1 mL IM)		X	X	
Hepatitis B (1 mL IM)		X	X	X
Meningococcal[d] (0.5 mL SQ)		X		
MMR [e] (0.5 mL SQ)				X
VZV [f] (0.5 mL SQ)				X

[a] Diphtheria and Tetanus Toxoids and Acellular Pertussis vaccine, Absorbed (DTaP)

[b] Haemophilus b Conjugate vaccine

[c] **Inactivated** Poliovirus vaccine

[d] Meningococcal polysaccharide vaccine (Menomune) is recommended for preadolescents (age 11-12) or other adolescents due to enter high school at 1 year post transplantation. A new meningococcal polysaccharide – protein conjugate vaccine (Menactra) is also available commercially but it is recommended that this product not be used at this time (Oct 2005) due to reported cases of Guillain-Barre Syndrome linked to the use of Menactra.

[e] Measles, Mumps, & Rubella vaccine, live, attenuated; only given if patient is immunocompetent at 24 months, i.e., NOT on immune suppression AND NO GVHD for six months.

[f] Varicella-Zoster Vaccine (VZV); only given if patients are immunocompetent at 24 months, i.e., NOT on immune suppression AND NO GVHD for six months.; If patients were vaccinated, *revaccinate*; If patients were never vaccinated with positive serology, *DO NOT vaccinate*; If patients were never vaccinated with negative serology, *vaccinate*; *Peds* ages 12 mo – 12 y receive a single dose; persons aged ≥ 13 y should receive two doses, 4–8 weeks apart.

11.2 APPENDIX B: MANAGEMENT OF ENGRAFTMENT SYNDROME

11.2.1 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 [105]:

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.

11.2.2 Treatment of Engraftment Syndrome

The mainstay of therapy for ES is high-dose corticosteroids, based upon the literature on diffuse alveolar hemorrhage [121] 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.*

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

11.3.1 Clinical Staging of Acute GVHD [102]

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.

11.3.2 Clinical Grading of Acute GVHD [102]

	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 ++++	+++

11.3.3 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) 1 mg/kg per dose IV, BID for 4 consecutive days.

2) If no response after 4 days, continue until response (7-day maximum trial); the dose may be doubled (4 mg/kg/day).

3) If response within 7 days, taper as follows:

   a) 0.75 mg/kg per dose IV BID for 2 days.

   b) 0.5 mg/kg per dose IV BID for 2 days.

   c) 0.375 mg/kg per dose IV BID for 2 days.

   d) If clinically appropriate, change MP to oral prednisone to 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% of starting oral dose 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 10 mg/kg 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/day of methylprednisolone (or equivalent) for $\geq$ 2 weeks. (See section 9.1.2.2) Voriconazole, caspofungin, liposomal amphotericin B (Ambisome), posaconazole or amphotericin B lipid complex (Abelcet) 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 or tacrolimus dose should be decreased by approximately 50%. In patients with therapeutic sirolimus levels at the initiation of voriconazole therapy, the sirolimus dose should be decreased by approximately 90%.

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:

Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD.

Partial Response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of adequate performance status (Karnofsky Score $\geq$ 70%).

Non-responder: < 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Failure to maintain adequate performance status (Karnofsky Score $\leq$ 70%).

Progressive disease: Further progression of signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

11.4 APPENDIX D – NMDP STANDARDS (20TH EDITION) AND NMDP STANDARD POLICIES AND PROCEDURES

(Please see attached).

11.5 APPENDIX E - NMDP PERIPHERAL BLOOD STEM CELL COLLECTION PROTOCOL

(Please see attached).

11.6 APPENDIX F - CHRONIC GVHD SCORE SHEET [57]

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: KPS ECOG LPS ☐	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement % BSA involved ☐	☐ No Symptoms	☐ <18% BSA with disease signs but NO sclerotic features	☐ 19-50% BSA OR involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA OR deep sclerotic features "hidebound" (unable to pinch) OR impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs with partial limitation of oral intake	☐ Severe symptoms with disease signs on examination with major limitation of oral intake

EYES Mean tear test (mm): □ >10 □ 6-10 □ ≤5 □ Not done	□ No symptoms	□ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) OR asymptomatic signs of keratoconjunctivitis sicca	□ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), WITHOUT vision impairment	□ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) OR unable to work because of ocular symptoms OR loss of vision caused by keratoconjunctivitis sicca

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

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

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

ĺ Esophageal stricture or web___	ĺ Pericardial Effusion___	ĺ Pleural Effusion(s)___
ĺ Ascites (serositis)___	ĺ Nephrotic syndrome___	ĺ Peripheral Neuropathy___
ĺMyasthenia Gravis___	ĺ Cardiomyopathy___	ĺ Eosinophilia > 500µl___
ĺ Polymyositis___	ĺ Cardiac conduction defects___	ĺ Coronary artery involvement___

ĺ Platelets <100,000/µl ___ĺ Progressive onset ____ Others_____

11.7 APPENDIX G - EXPERIMENTAL TRANSPLANTATION AND IMMUNOLOGY BRANCH PRECLINICAL SERVICE POLICY FOR SAMPLE HANDLING

11.7.1 Storage/Tracking

Normal donor and patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be archived by the ETIB Preclinical Service. All data associated with archived clinical research samples is entered into the ETIB Preclinical Services' Microsoft Excel databases on frozen cells and plasma. These databases are stored on the NCI group drive in the ETIB Preclinical Service folder. Access to this folder is limited to ETIB clinical staff, requiring individual login and password. All staff in the Preclinical Service laboratory have received annually updated NIH/CIT training and maintain standards of computer security.

The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, lymphapheresis, mobilized peripheral blood stem cells, marrow, pleural fluid) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples will receive a unique bar code number, which will be added to the sample Preclinical Service database. Only this bar code will be recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the Preclinical Service database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.

Samples are stored in locked freezers at -85°C (sera and plasma) or under liquid nitrogen (cells), according to stability requirements. These freezers are located onsite at the Preclinical Service laboratory (12C216) (-85° freezer) or in ETIB common equipment space (CRC/3-3273). Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol or through his/her submission and IRB approval of the NCI IRB Authorization Form (appended) stipulating whether IRB review is not necessary or IRB approval is granted for the pursuit of this new research activity. All researchers are required to sign a form (attached) stating that the samples are only to be used for research purposes associated with objectives of the original protocol for which the samples were collected, or (using only unlinked or coded samples) for an IRB approved protocol as stipulated on the IRB Authorization Form, and that any unused samples must be returned to the Preclinical Service laboratory.

11.7.2 Protocol Completion/Sample Destruction

Once primary research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or the IRB Authorization Form stipulating that the activity is exempt from IRB review (see attached authorization form from the NCI IRB).

Samples, and associated data, can only be permanently archived if the subject has provided informed consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the Preclinical Service laboratory.

The Preclinical Service staff will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher. The Principal Investigators will annually report this information to the IRB.

11.8 APPENDIX H: FLOW SHEET

*If Clinically Indicated

Observation	Eligibility within 28 days	Within 48 hrs Pre-induction	BiWk during induction	After each induction	Within 28 days pre- condition/Tx arm	48 hours pre - condition	Day 0--DOT	During Inpatient	Post Therapy Day 7	Post Therapy Day 14	Post Therapy Day 28	Post Therapy Day 42	Post Therapy Day 60	Day 100	Day 180	9 month	Post Therapy 12	Post Therapy 18	Post Therapy 24	Post Therapy 3, 4, 5 yrs
Clinical Note	X				X	X					X			X	X	X	X	X	X	X
NIH Advanced Directives Form (see section 7.3)[1]	X																			
Nutrition & Social Work Consult	X																			
Dental Consult	X												TMS (biopsy)	AC (biopsy)	X (biopsy)		X	X*	X*	X*
CMV, adenovirus, EBV, Toxo	X							Weekly Post transplant while on immunosuppression												
Ab for Hep A,B, C,HIV, HTLV1, Syphilis	X				X															
PPD	X*																			
ABO typing	X				X	X		ABO Q4 days												
Urinalysis & UPC	X				X	X								X			X			
24 Urine for Cr clearance	X	X*		X*		X														
Bone Marrow	X			FLAG & MM	X*						X*			X*	X*	X*	X*	X*	X*	X*
EKG	X				X															
CT chest, abd, pelvis & NECK*	X			lymphoma & CLL	X						X			X	X	X	X	X	X	X
CT/MRI head	X				X?															
CSF cytology, cell counts, chemistries, flow*	X*																			
PET scan for patients with Hodgkins and NHL*	X				X if disease presentat baseline						X			X	X	X	X	X	X	X
MM panel and workup	X			x	x						X			X	X	X	X	X	X	X

Observation	Eligibility within 28 days	Within 48 hrs Pre-induction	BiWk during induction	After each induction	Within 28 days pre- condition/Tx arm	48 hours pre - condition	Day 0--DOT	During Inpatient	Post Therapy Day 7	Post Therapy Day 14	Post Therapy Day 28	Post Therapy Day 42	Post Therapy Day 60	Day 100	Day 180	9 month	Post Therapy 12	Post Therapy 18	Post Therapy 24	Post Therapy 3, 4, 5 yrs
CBC with Diff, Acute Care Panel, Hepatitis Panel, Mineral Panel, PT, PTT	X	X	X	X	X	X		BID*		BiWk post transplant untilD+100	X	X	X	X	X	X	X	X	X	X
Urine/Serum Pregnancy	X	X			X	X														
PCR of DNA for future chimerism determination						X														
PFTs	X				X									X	X	X	X	X	X	X
ECHO	X				X									X	X*	X*	X*	X*	X*	X*
GVHD prophylaxis levels								2X per week												
Peripheral Blood Chimerism										X	X	X	X	x*	X*	X*	X*	PRN	PRN	PRN if no 100% chimerism
Bone Marrow Chimerism											X			X			X	PRN	PRN	PRN if no 100% chimerism
Acute GVHD Assessment										X	X	X	X	X	X	X	X	X	X	X
Chronic GVHD Assessment--NIH Staging														X	X	X	X	X	X	X
DEXA Scan																	X		yearly if abnornal	
Dermatology Consult													TMS (biopsy)	AC (biopsy)	X (biopsy)		X	X*	X*	X*
Ophthamology Consult	X																X		X*	X*
Gynecology Consult*	X*																X		X*	X*
FSH, LH (females) Testosterone Level (males only)																	X		X	X
TSH, Free T4, Ferritin, Vit D, Quantitative Immunoglobulins															X		X		X	X
TBNK	X			X		X			X	X	X		X	X	X	X	X	X	X	X
Research specimens	X	X		X		X	X		X	X	X		X	X	X	X	X	X	X	X

Observation	Eligibility within 28 days	Within 48 hrs Pre-induction	BiWk during induction	After each induction	Within 28 days pre- condition/Tx arm	48 hours pre - condition	Day 0--DOT	During Inpatient	Post Therapy Day 7	Post Therapy Day 14	Post Therapy Day 28	Post Therapy Day 42	Post Therapy Day 60	Day 100	Day 180	9 month	Post Therapy 12	Post Therapy 18	Post Therapy 24	Post Therapy 3, 4, 5 yrs
Immunizations															X*		X*	X*	X*	X (if catch up needed)

1. As indicated in section 7.3, all subjects ≥ age 18 will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

11.9 APPENDIX I: RISK OF RELAPSE ASSESSMENT

TRANSPLANTATION

Relapse risk in patients with malignant diseases given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning

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Allogeneic hematopoietic cell transplantation (HCT) after nonmyeloablative conditioning for hematologic malignancies depends on graft-versus-tumor effects for eradication of cancer. Here, we estimated relapse risks according to disease characteristics. Between 1997 and 2006, 834 consecutive patients (median age, 55 years; range, 5-74 years) received related (n = 498) or unrelated (n = 336) HCT after 2 Gy total body irradiation alone (n = 171) or combined with fludarabine (90 mg/m^2; n = 663). Relapse rates per patient year (PY) at risk, corrected for follow-up and competing nonrelapse mortality, were calculated for 29 different diseases and stages. The overall relapse rate per PY was 0.36. Patients with chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) in remission (CR), low-grade or mantle cell non-Hodgkin lymphoma (NHL) (CR + partial remission [PR]), and high-grade NHL-CR had the lowest rates (0.00-0.24; low risk). In contrast, patients with advanced myeloid and lymphoid malignancies had rates of more than 0.52 (high risk). Patients with lymphoproliferative diseases not in CR (except Hodgkin lymphoma and high-grade NHL) and myeloid malignancies in CR had rates of 0.26-0.37 (standard risk). In conclusion, patients with low-grade lymphoproliferative disorders experienced the lowest relapse rates, whereas patients with advanced myeloid and lymphoid malignancies had high relapse rates after nonmyeloablative HCT. The latter might benefit from cytoreductive treatment before HCT. (Blood. 2007;110:2744-2748)

Introduction

We have developed a nonmyeloablative regimen for allogeneic hematopoietic cell transplantation (HCT) for the treatment of patients with hematologic malignancies.[1-3] The regimen has been translated from a preclinical canine model and uses conditioning with 2 Gy total body irradiation (TBI) with or without fludarabine, and postgrafting immunosuppression with an antimetabolite, mycophenolate mofetil (MMF), and a calcineurin inhibitor, cyclosporine (CSP).[4] The latter 2 drugs are given for the dual purposes of enhancing engraftment and controlling graft-versus-host disease (GVHD). The regimen relies virtually entirely on graft-versus-tumor effects for eradicating cancer and can largely be administered in the ambulatory care setting because of the lack of serious regimen-related toxicities. The latter characteristic has enabled us to loosen the age and comorbidity limitations currently existing for myeloablative regimens.[1,5,6] Given that and the fact that median ages at diagnosis for patients with most candidate diseases range from 65 to 70 years, the number of patients treatable by allogeneic HCT has been greatly increased. It is noteworthy that the regimen allows for the purest determination of graft-versus-tumor effects apart from conditioning and, therefore, provides an excellent foundation on which to add disease and disease stage–specific modalities (eg, targeted radiotherapy or cytoreductive autografts). The aim of the current retrospective analysis was to estimate relapse risks after HCT according to pretransplant disease characteristics and define groups of patients with low, standard, or high risks for relapse after HCT.

Patients, materials, and methods

Eligibility criteria

Between December 17, 1997, and June 30, 2006, 834 patients with various hematologic malignancies were treated under different nonmyeloablative transplantation protocols within a consortium, consisting of the Fred Hutchinson Cancer Research Center (FHCRC), the University of Washington, Seattle Veterans Affairs Medical Center, Childrens Hospital and Regional Medical Center, all in Seattle, WA; Rocky Mountain Blood and Marrow Transplant Program, Denver, CO; Stanford University, Stanford, CA; the University of Leipzig, Leipzig, Germany; City of Hope National Medical Center, Duarte, CA; Baylor University, Dallas, TX; Oregon Health and Science University, Portland, OR; the University of Torino, Torino, Italy; Emory University, Atlanta, GA; University of Utah Health Sciences Center and LDS Hospital, Salt Lake City, UT; Medical College of Wisconsin, Milwaukee, WI; and the University Hospital Tübingen, Tübingen, Germany. FHCRC served as the coordinating center for all

Submitted March 16, 2007; accepted June 4, 2007. Prepublished online as *Blood* First Edition Paper, June 26, 2007; DOI 10.1182/blood-2007-03-078592.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Table 2. Relapse rates in 29 diagnosis and disease stage groups

Disease	Disease stage	Patients, N	PY of follow-up*	Relapse rate per PY
Low risk				
CLL	CR	7	8.2	0.00
NHL, low grade	Not in CR	34	40.8	0.15
NHL, low grade	CR	9	11.1	0.18
Waldenström	Advanced	9	10.8	0.19
MM	CR†	29	42.8	0.19
NHL, mantle cell	CR	16	15.7	0.19
NHL, mantle cell	Not in CR	25	30.4	0.20
MPD	Advanced	19	18.6	0.21
NHL, high grade	CR	26	31.0	0.23
ALL	1st CR	19	21.0	0.24
Standard risk				
CLL	Not in CR	75	89.0	0.26
MM	Not in CR	136	174.8	0.27
MDS	RA/RARS	20	18.2	0.33
AML	1st CR	80	78.3	0.33
CML	1st CP	26	32.8	0.34
AML	≥2nd CR	59	62.6	0.37
High risk				
MDS	RAEB/RAEB-t	23	19.2	0.52
AML	Evolved from MDS	42	29.1	0.55
NHL, high grade	Not in CR	36	26.3	0.57
HD	CR	13	9.6	0.62
MDS	Secondary	18	14.4	0.70
HD	Not in CR	38	30.6	0.72
AML	Not in CR	13	9.2	0.87
CML	AP/BC	14	10.1	0.99
CML	2nd CP	7	3.8	1.05
Renal cell	Metastatic	18	10.6	1.23
ALL	≥2nd CR	8	4.6	1.29
ALL	Not in CR	3	2.2	1.35
CMML	Advanced	12	6.3	1.42

CLL indicates chronic lymphocytic leukemia; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; CR, complete remission; MPD, myeloproliferative disease; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CP, chronic phase; RAEB, refractory anemia with excess blasts; RAEB-t, refractory anemia with excess blasts in transformation; HD, Hodgkin disease; AP, accelerated phase; BC, blast crisis; CMML, chronic myelomonocytic leukemia.

* PY of follow-up is the total person-years of observation time from transplant until death, relapse/progression, last contact, or 2 years.

† The criteria for complete remission from MM were absence of monoclonal immunoglobulin and of discernible light chains in urine by standard electrophoresis, the absence of visible monoclonal bands on immunofixation, <1% plasma cells in marrow aspirates, the absence of evidence of clonal disease according to flow cytometry of marrow cells, and the absence of an increase in the size or number of osteolytic lesions.

CR (relapse rate per PY, 0.23); and acute lymphoblastic leukemia (ALL) in CR1 (relapse rate per PY 0.24).

The standard-risk group included patients with CLL or MM with measurable disease at HCT (relapse rates per PY, 0.26 and 0.27, respectively; 94 of the 136 patients with MM had preceding autologous HCT after high-dose melphalan[7]); myelodysplastic syndromes (MDS)-refractory anemia (RA)/refractory anemia ringed sideroblasts (RARS), AML in first CR, CML in first chronic phase (CP), and AML in second or later CR (relapse rates per PY ranging from 0.33 to 0.37).

The high-risk group included patients with myelodysplastic syndrome (MDS)-refractory anemia with excess of blasts (RAEB)/RAEB in transformation (RAEB-t), AML after MDS or AML not in CR, high-grade NHL not in CR, Hodgkin lymphoma, MDS after chemotherapy, CML in second CP or accelerated phase/blast crisis, ALL in second or later CR, chronic myelomonocytic leukemia (CMML), and renal cell carcinoma, with relapse rates per PY ranging from 0.52 to 1.42.

Figure 1 illustrates the impact of relapse risk on overall survival. Three-year survival for low-risk patients was 60% compared with 55% for those with standard risk and 26% for those in the high-risk group (Figure 1A). The survival differences were largely due to differences in relapse mortality (Figure 1B). The cumulative relapse incidences at 3 years were 25% for low-risk, 40% for standard-risk, and 57% for high-risk patients. In contrast, no significant differences in nonrelapse mortalities within the 3 risk groups were seen (Figure 1C). The cumulative nonrelapse mortality incidences at 3 years were 29% for low-risk, 21% for standard-risk, and 26% for high-risk patients.

Discussion

The success of allogeneic HCT in curing patients with hematologic malignancies depends, in part, on cytotoxic antitumor effects of

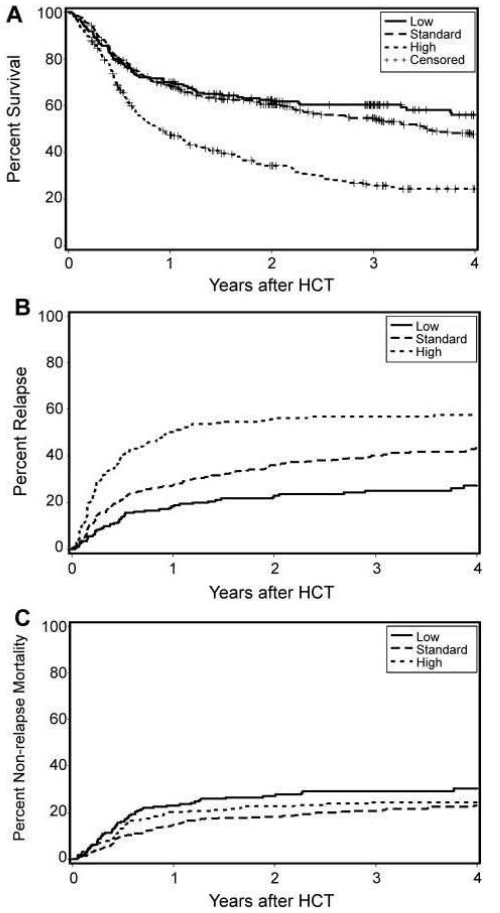

Figure 1. Overall survivals (A), cumulative relapse rates (B), and cumulative nonrelapse mortality rates according to risk groups (C).

conditioning regimens and, in part, on immune-mediated destruction of cancer cells through grafted cells. Target antigens for T-cell–mediated graft-versus-tumor effects can be ubiquitous polymorphic minor histocompatibility antigens and/or antigens uniquely expressed on hematopoietic cells (hematopoietic antigens),[8,9] although a role for natural killer cells has also been postulated.[10] The current conditioning regimen of 2 Gy TBI with or without fludarabine was designed to reduce toxicities and allow extending HCT to older and sicker patients and, therefore, has few cytotoxic antitumor effects. As a result, tumor eradication relies virtually entirely on graft-versus-tumor activities. These, in turn, can vary in intensity, depending on the immunogenicity of the tumors and the respective proliferation rates both of the tumors and the donor immune cells poised to destroy them.

The current study analyzed outcomes in 834 consecutive patients with various hematologic malignancies given either related or unrelated HCT and sought to determine which diseases and disease stages responded well and which less well to graft-versus-tumor effects as assessed by relapse rates per PY. Using this criterion, patients could be roughly divided into 3 groups that had either low, standard, or high risks of relapse, although nonrelapse mortalities among the 3 groups were comparable, the latter ranging from 21% to 29% at 3 years. Accordingly, differences in survival among the 3 groups were largely determined by differences in relapse rates. These ranged from as low as 0-0.24 relapses per PY in the low-risk group to 0.52-1.42 in the high-risk group, with the standard-risk patients placed in between (0.26-0.37). Three-year survival rates were 60% for the low-risk group, 55% for the standard-risk group, and 26% for the high-risk group.

The low-risk group included patients with lymphoid malignancies who were in CR (eg, CLL, ALL in CR1, Waldenström's, MM, and high-grade, mantle cell, and low-grade NHL [the latter 2 also included patients in PR]). The low tumor burden and slow-growing nature of these cancers combined with their presumed ability to present target antigens to the donor T cells were likely reasons for the low relapse rates and good posttransplantation survival. Similarly good outcomes in these diseases and disease stages have also been reported by others, with progression-free and overall survival rates ranging from 29.4% to 84% and 41% to 84%, respectively, although, as a rule, the conditioning regimens used were more intense. For example, Schetelig et al[11] used a combination of fludarabine, busulfan, and antithymocyte globulin for conditioning. Dreger et al[12] reported on various regimens, including fludarabine/cyclophosphamide and low-dose TBI; Khouri et al[13] combined fludarabine with cyclophosphamide; Lee et al[14] conditioned either with 100 mg melphalan/m^2 (related donors) or melphalan/250 cGy TBI/fludarabine (unrelated donors); and Gerull et al[15] used fludarabine/low-dose TBI, similar to the current study. Patient ages in these studies were slightly, but not significantly, lower than current patients. Donors were more often related than unrelated. Current results suggested that a minimal conditioning regimen that enabled sustained allogeneic engraftment might be sufficient in patients with low-grade B-cell malignancies in CR or PR.

The standard-risk group included patients with MM and CLL not in CR, and various early-stage myeloid malignancies. Although the balance was still in favor of the grafted donor immune cells, with the result that a majority of patients either achieved CR or remained in CR, it was clear that larger tumor burden, relatively faster proliferation rates of tumor cells, and perhaps variable sensitivity of cancer cells to donor immune cells adversely affected outcomes in a strong minority of patients who experienced relapse/progression. To improve outcomes and avoid across-the-board increases in conditioning intensity (and toxicity), individual patients at risk of relapse need to be identified. For example, in the case of patients with CML in CP1, relapse/recurrence was a direct consequence of an initially observed high rate of nonfatal graft rejections, and this problem has since been addressed by adding fludarabine to 2 Gy TBI or increasing the TBI dose to 3 Gy. In patients with CLL who had bulky lymphadenopathy and thus were at high risk of relapse, a single dose of a radiolabeled monoclonal antibody to CD20 has been added to the conditioning regimen.[16] For other disease groups and stages, pinpointing risk factors might require larger numbers of patients, although the fact that almost half of the patients with AML in CR1 and CR2 were older than 60 years might have contributed to a higher relapse risk. Others have used a more intensive conditioning regimen of 8 Gy TBI plus fludarabine, and reported 60%-70% relapse-free survivals in a slightly younger cohort of patients with AML in first or second remission, supporting the notion that more intensive conditioning might be beneficial in some patients.[17] Nevertheless, their reported survival of patients with more advanced AML was not better than that observed among a similar group of patients reported here. Data comparable with ours were described by Gupta et al,[18] using fludarabine and 2 Gy TBI, and Sayer et al,[19] using fludarabine and reduced doses of busulfan both in patients with AML who were in morphologic CR at HCT and those with more advanced disease. Khouri et al[20] observed 83% 2-year survival in patients with CML in chronic phase conditioned with 550 cGy TBI (delivered at 35 cGy/min), a result that was comparable with the survival of those current patients who were conditioned with fludarabine and 2 Gy TBI.

The high-risk group was comprised of patients with advanced stages of NHL (not in CR), Hodgkin lymphoma, MDS, CML, CMML, acute leukemias, and renal cell carcinoma. Here, the large numbers of cancer cells present at HCT might have shifted the balance in their favor, and they "outproliferated" the cytotoxic donor immune cells in a majority of patients. Cytotoxic donor immune cells tended to work slowly, and even under the best of circumstances, say, in patients with slow growing B-cell malignancies such as CLL, graft-versus-tumor effects might take many months before accomplishing molecular remissions.[21] It remains to be seen whether adding targeted therapy to the current regimen, including radiolabeled monoclonal antibodies to CD20[16] or CD45[22] expressed on tumor cells, will reduce the tumor burden sufficiently to tilt the balance toward the donor immune cells. Alternatively, for patients with MDS, a dose escalation of TBI to 3 Gy is being explored, in part to decrease the relatively high rate of graft failure. A strong hint that increasing the intensity of the conditioning regimen might improve outcomes comes from a study in patients with Hodgkin lymphoma; in that study, patients were conditioned with fludarabine, melphalan, and alemtuzumab, and had 4-year overall and progression-free survivals of 55.7% and 39%, respectively.[23]

In conclusion, allogeneic graft-versus-tumor effects are powerful and can lead to cures of otherwise incurable hematologic malignancies. They work best in patients with relatively low tumor burdens and slow growing tumors (eg, NHL or CLL) and least well in patients who have bulky tumors with relatively fast proliferation rates (eg, acute leukemias in relapse). To improve outcomes in such patients, either the option of allografting should be considered earlier in the disease course when the tumor burden is lower, or targeted therapies with limited systemic toxicities should be added,

such as radiolabeled monoclonal antibodies directed against surface antigens that are specific for the tumor cells.

Acknowledgments

We are grateful to the patients and their donors who participated in this study. In addition, we thank all physicians, nurses, and support personnel for their very dedicated care of patients on this study, the research nurses John Sedgwick, Mary Hinds, Michelle Bouvier, and data coordinator Heather Hildebrandt for their help in data acquisition, the long-term follow-up team, and Helen Crawford and Bonnie Larson for manuscript preparation.

This work was supported by National Institutes of Health grants CA78902, CA18029, CA15704, CA49605, CA30206, DK064715, and HL36444. C.K. was supported by a fellowship from Deutsche Krebshilfe, Dr Mildred-Scheel-Stiftung für Krebsforschung. B.B. received support from the Fondazione Cassa di Risparmio di Torino and Comitato Regionale Piemontese Gigi Ghirotti, Progetto Vita Vitae.

Authorship

Contribution: C.K. performed retrospective data collection and drafted the manuscript. B.E.S. designed research, performed statistical analyses. B.M.S. and D.G.M. designed research protocol, entered patients on study, verified data, and participated in the writing of the manuscript. M.M. verified data and assisted in drafting the manuscript. M.B.M., K.G.B., D.N., T.R.C., S.J.F., E.A., J.F.L., B.B., A.L., P.A.M., J.C.W., E.E., F.B.P., and W.A.B. contributed patients to studies, verified patient data, and reviewed the manuscript. R.S. designed, directed, and provided funding for the study and edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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11.10 APPENDIX J: COMOBIDITY INDEX

blood

2005 106: 2912-2919
Prepublished online June 30, 2005;
doi:10.1182/blood-2005-05-2004

Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT

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Hematopoietic cell transplantation (HCT)–specific comorbidity index: a new tool for risk assessment before allogeneic HCT

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We previously reported that the Charlson Comorbidity Index (CCI) was useful for predicting outcomes in patients undergoing allogeneic hematopoietic cell transplantation (HCT). However, the sample size of patients with scores of 1 or more, captured by the CCI, did not exceed 35%. Further, some comorbidities were rarely found among patients who underwent HCT. Therefore, the current study was designed to (1) better define previously identified comorbidities using pretransplant laboratory data, (2) investigate additional HCT-related comorbidities, and (3) establish comorbidity scores that were suited for HCT. Data were collected from 1055 patients, and then randomly divided into training and validation sets. Weights were assigned to individual comorbidities according to their prognostic significance in Cox proportional hazard models. The new index was then validated. The new index proved to be more sensitive than the CCI since it captured 62% of patients with scores more than 0 compared with 12%, respectively. Further, the new index showed better survival prediction than the CCI (likelihood ratio of 23.7 versus 7.1 and *c* statistics of 0.661 versus 0.561, respectively, $P < .001$). In conclusion, the new simple index provided valid and reliable scoring of pretransplant comorbidities that predicted nonrelapse mortality and survival. This index will be useful for clinical trials and patient counseling before HCT. (Blood. 2005;106: 2912-2919)

Introduction

Comorbidity, as defined by Feinstein,[1] is any distinct additional clinical entity that has existed or may occur during the clinical course of a patient with a primary (index) disease. Comorbidities affect therapeutic plans and posttherapeutic outcomes of the index disease.[1] In patients with cancer as the index disease, multiple studies have demonstrated the relevance of comorbidities in the prognosis (reviewed in Extermann[2]). The number of comorbidities was suggested to increase with aging of cancer patients (reviewed in Extermann[3]). In Surveillance, Epidemiology, and End Results (SEER) registry data, 75% of patients 55 years and older with colon cancer had more than one comorbidity.[4] However, patients with comorbidities are often excluded from clinical trials, and there are little data on how to translate results from cooperative studies to patients with comorbid diseases. Therefore, the assessment of comorbidities should be integrated into clinical trials, in addition to functional status, particularly since comorbidities and functional status can be used independently to predict outcomes.[5-7] Efforts to analyze the impact of comorbidities on index diseases are still in their early stages. The interactions between the index diseases and different comorbidities are variable based on the type and degree of organ involvement. Therefore, several indices have introduced a way to rate the impact of different comorbidities on the index disease. One of these is the Charlson Comorbidity Index (CCI). The CCI was developed by assigning weights for 19 chronic conditions according to their association with 1-year mortality in a cohort of 559 patients admitted to a general medical center. Then, a summary score based on the sum of the weights was validated in a cohort of breast cancer patients by evaluating the ability of scores to predict mortality.[8] Later, Charlson added age to her weighted comorbidity scores by assigning an extra point for every decade of age starting at 50 years.[9] The CCI has been widely used in predicting mortality risks in various medical conditions including solid malignancies.[3,10-17] We have recently used the CCI in the settings of ablative and nonablative allogeneic hematopoietic cell transplantation (HCT) for patients with hematologic malignancies. In our studies, an adapted form of the CCI successfully predicted the risks of nonrelapse mortality (NRM) in patients receiving hematopoietic cell transplants from unrelated[18] or related[19] donors. Those initial observations have been confirmed in several subsequent reports analyzing outcomes after nonmyeloablative HCT.[20-23] However, some of the comorbidities described by Charlson were rarely encountered in HCT patients due to existing exclusion

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Submitted May 19, 2005; accepted June 16, 2005. Prepublished online as *Blood* First Edition Paper, June 30, 2005; DOI 10.1182/blood-2005-05-2004.

Supported in part by grants CA78902, CA92058, CA18029, CA49605, HL36444, and CA15704 from the National Institutes of Health, Department of Health and Human Services (DHHS), Bethesda, MD. M.L.S. was supported by a grant from the Oncology Research Faculty Development Program of the Office of International Affairs of the National Cancer Institute.

M.L.S. designed the study, collected and extracted patients' data, performed the research, analyzed the statistical results, and wrote the paper. M.B.M. provided guidance for the study design, contributed to analysis of the statistical results, and edited the paper. R.S. provided guidance for the study design, has been senior investigator on underlying clinical studies, contributed to analysis of the statistical results, provided oversight, and edited the paper. F.B. contributed to collection of patient data and edited the paper. B.M.S. provided guidance for the study design and edited the paper. D.G.M. provided guidance for the study design and edited the paper. B.S. contributed to the statistical design of the study, performed the statistical analyses, contributed to writing "Patients, materials, and methods," and edited the paper.

An Inside *Blood* analysis of this article appears at the front of this issue.

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criteria. This was particularly true for hepatic and pulmonary comorbidities. In addition, the CCI did not capture some frequent comorbidities, such as recent infections and psychiatric disturbances. Further, the CCI lacked sensitivity since comorbidities were found in only 35% of patients, and this percentage was even lower in patients undergoing ablative HCT (12% in unrelated[18] and 22% in related[19] ablative recipients). Some investigators have pointed out the somewhat limited ability of the CCI to capture comorbidities in patients with primary diagnoses other than hematologic diseases.[5,24] Others were successful in developing disease-specific comorbidity indices by modifying the original CCI.[12,13] With the development of nonmyeloablative HCT regimens and improvements in the supportive care after myeloablative HCT regimens, more patients with comorbidities are being offered allogeneic HCT. Therefore, in the current study, we sought to identify those chronic medical conditions, which were important in predicting NRM in HCT patients, and then integrate these factors into a new scoring system to assess survival probabilities after allogeneic HCT.

Patients, materials, and methods

The analysis was approved by the institutional review board of the Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA). Informed consent was provided according to the Declaration of Helsinki.

Patients: data source and patient selection

This analysis included consecutive and concurrent patients given either nonablative (n = 294) or ablative (n = 761) conditioning followed by hematopoietic cell grafts from related (1997-2003) or unrelated (2000-2003) donors. Nonablative patients were conditioned with 2 Gy total body irradiation (TBI) either alone (n = 68) or preceded by fludarabine (n = 226) and were given postgrafting immunosuppression with cyclosporine (CSP) and mycophenolate mofetil (MMF).[25-28] Ablative patients were conditioned with either busulphan/cyclophosphamide[29] or cyclophosphamide/TBI[30,31] and, in almost all cases, were given postgrafting immunosuppression with CSP/methotrexate.[32]

Diagnoses and clinical grading of acute and chronic graft-versus-host disease (GVHD) were performed using standard criteria.[33,34] Primary treatment of GVHD consisted of systemic corticosteroids, oral beclomethasone with or without systemic corticosteroids, or reinstitution of CSP.

Early detection of cytomegalovirus antigenemia and preemptive ganciclovir therapy were used for all patients[35] as were standard prophylaxis against *Candida* infections (fluconazole),[36] bacterial infections (ceftazidime or ciprofloxacin), *Pneumocystis carinii* infection (trimethoprim-sulfamethoxazole or dapsone),[37] and herpes simplex virus[38] and varicella zoster virus[39] reactivation in serologically positive patients (acyclovir).

Low disease risk included acute leukemia in first complete remission, chronic myeloid leukemia in first chronic phase, myelodysplasia-refractory anemia, and nonmalignant hematologic diseases; while high disease risk included all other diagnoses.

Data collection and modified comorbidities

Demographic data were obtained from the FHCRC database. Information on comorbidities was extracted from detailed review of the patients' medical charts and laboratory values at the time of HCT. All comorbidities, which were encountered in the 1055 reviewed patients, were included in the current study. Patients were given scores based on the original CCI.[8] A new HCT-specific comorbidity index (HCT-CI) was then developed as follows: First, definitions of several comorbidities were modified as described in Table 1. Second, comorbidities that were not found in the original CCI were identified as newly investigated or additional comorbidities. Those included bleeding, headache, osteoarthritis, osteoporosis, asthma, obesity, infection, and psychiatric disturbances. The added comorbidities had to be active or requiring treatment at the time of HCT, as defined by Charlson et al.[8] Third, all other comorbidities were assigned new weights in the HCT-CI using the original definitions in the CCI,[8] including gastrointestinal disease, coagulopathy, endocrine disease, hypertension, arrhythmia, inflammatory bowel disease, diabetes, cerebrovascular disease, rheumatologic disease, peptic ulcer, and heart valve disease.

Assignment of new comorbidity weights

In order to develop the new HCT-CI, patients were randomly divided into 2 cohorts. Two thirds of the patients were assigned to a training set to develop the scoring weights (n = 708), and one third was assigned to a validation set (n = 347). Integer weights for the HCT-CI were derived from Cox proportional hazards modeling applied to the training set, with NRM over the first 2 years as the outcome. NRM was used instead of survival for development of the scores, since deaths from nonrelapse causes are more likely influenced by pretransplant comorbidities than deaths caused by disease progression or relapse, which were treated as competing risks. Adjusted hazard ratios (HRs) for NRM over the first 2 years after transplantation were calculated for each comorbid condition, controlling for the presence of all coexisting comorbidities as well as for age (younger versus older than 50 years), type of conditioning (nonablative versus ablative), and disease risk (high versus low). The adjusted HRs were converted to integer weights according to the following: comorbidities with adjusted HR of 1.2 or less were dropped from consideration, comorbidities with adjusted HR of 1.3 to 2.0 were assigned a weight of 1, comorbidities with adjusted HR of 2.1 to 3.0 were assigned a weight of 2, and

Table 1. Refinement of definitions of comorbidities

Comorbidity	CCI definition	New definition, from HCT-CI
Mild pulmonary	Dyspnea on moderate activity	Dyspnea on moderate activity or DLco and/or FEV_1 81%-90%
Moderate pulmonary	Dyspnea on slight activity	Dyspnea on slight activity or DLco and/or FEV_1 66%-80%
Severe pulmonary	Dyspnea at rest or requires oxygen	Dyspnea at rest or requires oxygen or DLco and/or $FEV_1 \leq 65\%$
Cardiac	Congestive heart failure (symptomatic and requiring treatment) and myocardial infarction were included as independent comorbidities, each acquiring a score of 1	Includes coronary artery disease,* congestive heart failure, myocardial infarction, or ejection fraction ≤ 50%: one or more acquiring a score of 1
Mild hepatic	Chronic hepatitis or cirrhosis	Chronic hepatitis, bilirubin > ULN to 1.5 × ULN, or AST/ALT > ULN to 2.5 × ULN
Moderate-severe hepatic	Cirrhosis with portal hypertension ± bleeding varices	Cirrhosis, fibrosis, bilirubin > 1.5 × ULN, or AST/ALT >2.5 × ULN
Mild renal	Serum creatinine 2-3 mg/dL	Creatinine 1.2-2 mg/dL
Moderate-severe renal	Creatinine > 3 mg/dL, renal dialysis, or renal transplant	Creatinine > 2 mg/dL, renal dialysis, or renal transplant
Prior solid tumor	Initially treated in the last 5 y	Treated at any time point in the patient's past history, excluding nonmelanoma skin cancer

To convert creatinine from milligrams per deciliter to micromoles per liter, multiply milligrams per deciliter by 88.4.

*One or more vessel-coronary artery stenosis requiring medical treatment, stent, or bypass graft.

DLco indicates diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; ULN, upper limit of normal; AST, aspartate aminotransferase; and ALT, alanine aminotransferase.

comorbidities with adjusted HR of 3.1 or more were assigned a weight of 3. The HCT-CI score was the sum of these integer weights. The HCT-CI scores were further collapsed into 3 risk groups: 0 (low risk), 1 to 2 (intermediate risk), and 3 or more (high risk). For comparative purposes, the original CCI was also reduced to 3 risk groups: 0, 1, and 2 or more.

Model validation

The scores of the new HCT-CI were applied to patients of the validation set to test their ability to predict for HR and cumulative incidence of NRM. The new HCT-CI was then compared with the original CCI for prediction of both NRM and survival. Survival was added as an end point for comparison between the 2 indices since the CCI scores were derived from Cox proportional hazards modeling for survival.[8] Cumulative incidence[40] curves for NRM and Kaplan-Meier curves for survival were computed for each risk group defined by the 2 indices. Likelihood ratio statistics from proportional hazards models were computed for both indices for events over the first 2 years. Although a larger likelihood ratio statistic indicated a better fit to the data, the likelihood ratio by itself had no intuitive interpretation in terms of prediction. For this purpose, we also computed the *c*-statistic.[41] For a continuous predictor, the *c*-statistic could be interpreted as the probability that a random pair of observations whose event times could be ordered would have a concordant ordering of the predictor. A value of 1.0 indicated perfect predictive discrimination, whereas a value of 0.5 indicated no ability to discriminate. If the data were collapsed to indicate a binary outcome (survival larger or smaller than a fixed point in time), then the *c*-statistic was also interpretable as the area under a receiver operating characteristic curve. Pairs of observations whose ordering could not be assigned because of censoring were excluded from the calculation. The *c*-statistic was computed for NRM and survival, based on both time to event over the first 2 years and as a binary outcome indicating event times before/after 1 year or 2 years. For computing *c*-statistic for NRM, patients were censored at the time of relapse/progression. Standard errors for the *c*-statistic were estimated by applying a bootstrap procedure to the validation dataset, using 100 bootstrap samples. Similarly, the standard error for the difference in *c*-statistic between the CCI and HCT-CI was estimated from the bootstrap samples and used to calculate a z-score and *P* value for the difference.

Results

Patient characteristics

Characteristics of the 1055 patients included in this study are shown in Table 2. A majority of patients received cyclophosphamide/busulphan conditioning (43%), 29% received cyclophosphamide/TBI (12-14.4 Gy), 22% received fludarabine/TBI (2 Gy), and 6% received 2 Gy TBI. GVHD prophylaxis was MTX/CSP in 69% of patients and MMF/CSP in 31%. Myeloid malignancies constituted the majority of diagnoses (66%). Forty-one percent of the patients had high risk disease. Median age was 44.8 years. Related grafts were used in 58% of patients, while the remainder received unrelated grafts. Thirteen percent of patients had preceding myeloablative HCT, of which 2% were allogeneic, 6% were failed autologous, and 5% were planned autologous. A majority of patients received peripheral blood stem cell grafts (71%).

The HCT-CI

The prevalence of various comorbidities together with the cumulative incidence and multivariate HR for 2-year NRM, as predicted by each comorbidity, are shown in Table 3. The definitions of comorbidities included in the HCT-CI, along with the integer weights compared with those of the CCI, are shown in Table 4. Pulmonary and hepatic abnormalities, as defined by laboratory data, were the most frequent comorbidities in the new HCT-CI and, together with prior solid tumor and heart valve disease, had the highest assigned weights. By comparing conditions in the newly derived HCT-CI with those of the CCI, a number of comorbidities increased in relative importance, including arrhythmia, inflammatory bowel disease, rheumatologic disease, peptic ulcer, moderate and severe pulmonary comorbidity, prior solid tumor, and heart valve disease. Other conditions, including cardiac disease, diabetes mellitus, cerebrovascular disease, mild hepatic comorbidity, moderate/severe renal comorbidity, and moderate/severe hepatic comorbidity, had comparable weights both in the HCT-CI and the original CCI. Of the 8 comorbidities tested that were not specified by Charlson, obesity, peritransplant infections, and psychiatric disturbances were assigned weights in the HCT-CI, whereas bleeding, headache, osteoarthritis, osteoporosis, and asthma were not because of low predictive HR for NRM (0.0, 0.3, 0.4, 0.7, and 1.1,

Table 2. Patient and disease characteristics

Characteristic	Data
Conditioning regimens, %	
2 Gy TBI	6
Fludarabine + 2 Gy TBI	22
CY + at least 12 Gy TBI	29
BU + CY	43
Postgrafting immunosuppression, %	
CSP + MMF	18
CSP + MTX	82
Donor type, %	
Related donor	58
Unrelated donor	42
Diagnoses, %	
AML	27
CML	20
MDS	19
ALL	10
NHL	9
MM	6
CLL	4
HD	2
Nonmalignant hematologic*	3
Disease risk group, %†	
High	41
Low	59
Age at transplantation	
Median (range), y	44.8 (0.8-72.7)
Preceding myeloablative HCT, %	
Allogeneic	2
Failed autologous	6
Planned autologous	5
Hematopoietic cell source, %	
G-PBMC	71
Marrow	29
Male/female, %	
Patients	56/44
Donors	51/49

n = 1055.

TBI indicates total body irradiation; CY, cyclophosphamide; BU, busulfan; CSP, cyclosporine; MMF, mycophenolate mofetil; MTX, methotrexate; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndromes; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; CLL, chronic lymphocytic leukemia; HD, Hodgkin disease; HCT, hematopoietic cell transplantation; and G-PBMC, granulocyte colony stimulating factor-mobilized peripheral blood mononuclear cells.

*Immunodeficiency syndrome, chronic granulomatous disease, congenital dyserythropoietic anemia, paroxysmal nocturnal hemoglobinuria, polycythemia vera, thalassemic syndrome, and sickle cell anemia.

†Low indicates acute leukemia in first remission, CML in first chronic phase, MDS-refractory anemia, or nonmalignant hematologic disease; while high indicates all other diagnoses.

Table 3. Prevalence of comorbidities among 708 patients included in the training set with cumulative incidences and multivariate HRs of comorbidities for 2-year NRM

Comorbidity	Prevalence, %	2-year NRM, %	Multivariate HR*
Bleeding†	1	0	0.0
Headache†	4	8	0.3
Osteoarthritis†	1	11	0.4
Gastrointestinal disease	9	20	0.5
Coagulopathy	6	30	0.6
Osteoporosis†	1	75	0.7
Renal, mild‡	10	24	0.8
Endocrine disease	5	31	1.0
Asthma†	2	14	1.1
Pulmonary, mild‡	16	18	1.1
Hypertension	10	33	1.2
Arrhythmia	5	35	1.3
Cardiac‡	5	37	1.3
Inflammatory bowel disease	1	31	1.3
Diabetes	3	50	1.6
Cerebrovascular disease	< 1	67	1.6
Psychiatric disturbance†	9	29	1.8
Hepatic, mild‡	16	30	1.9
Obesity†	2	33	1.9
Infection†	4	40	1.9
Rheumatologic	4	45	2.3
Peptic ulcer	1	38	2.5
Renal, moderate/severe‡	2	42	2.6
Pulmonary, moderate‡	24	36	3.0
Prior solid tumor‡	2	38	3.1
Heart valve disease	2	33	3.3
Pulmonary, severe‡	9	39	3.7
Hepatic, moderate/severe‡	4	43	3.9
Peripheral vascular disease	0	—	—

— indicates not applicable.

*Adjusted for all other comorbidities, age, high disease risk, and conditioning type.

†Newly investigated comorbidities.

‡Comorbidities with modified definitions compared with the original CCI.

respectively). For peripheral vascular disease, no conclusions could be drawn since none of the current patients had this comorbidity at the time of HCT.

The HCT-CI scores ranged from 0 to 11 in the training set, compared with 0 to 4 for the original CCI scores.

Validation of the new HCT-CI

The newly developed HCT-CI scores were then calculated for patients in the independent validation set (n = 347). The HR and 2-year cumulative incidence of NRM are summarized by HCT-CI scores for the training and validation sets in Table 5. Due to small numbers, patients with scores of 4 or more were collapsed into one group. Probably due to the smaller number of patients, there was an apparent shrinkage of the discriminatory ability for NRM among various risk groups between the training and validation sets; nevertheless, the HCT-CI continued to be strongly associated with NRM.

Comparing the new HCT-CI with the original CCI

For the purpose of comparison, the 2 comorbidity indices were each separated into 3 risk groups. For the HCT-CI these were low (0), intermediate (1-2), and high (≥ 3) and for the CCI, low (0), intermediate (1), and high (≥ 2). Results for NRM and survival are summarized by risk group in Table 6 and Figure 1. It was clear that the risk groups based on the HCT-CI scores were more evenly distributed and provided better discrimination of NRM risks than the risk groups defined by the CCI. Although the risk groups for both indices provided some discrimination for survival, the CCI had a lower overall predictive value for survival because of the concentration of nearly 90% of scores in the 0 score category.

Statistical comparisons between the HCT-CI and CCI are summarized in Table 7. There was stronger association of higher HCT-CI scores with worse NRM and survival based on the likelihood ratio. In addition, the *c*-statistics were significantly higher using the HCT-CI compared with the CCI, whether as an overall measure of association (the percent of pairs of patients where the patient with the higher comorbidity score failed first) or for association with a dichotomized end point at 1 year or 2 years (the percent of pairs of patients where the patient failing before 1 year or 2 years had a higher comorbidity score than the patient failing after that time).

Correlation between causes of death and most common comorbidities

Pulmonary comorbidities, as defined by impairments of forced expiratory volume in one second (FEV_1) and/or diffusion capacity of carbon monoxide (DLco), and hepatic comorbidities, as defined by elevations of hepatic function tests, were the most prevalent comorbidities captured by the new HCT-CI. There were 347 patients with moderate (score 2) or severe (score 3) pulmonary comorbidities, and 122 died from nonrelapse causes. Causes of NRM among these 122 patients included pulmonary toxicities (24%), GVHD (11%), and infections (45%) with (18%) or without (27%) GVHD (infections involved the lungs in almost half of the cases), and 20% died from other causes.

Mild (score 1) and moderate-severe (score 3) hepatic comorbidities were encountered in 215 patients, of whom 72 died of nonrelapse causes. Twenty-one percent of these 72 patients died from GVHD; 10%, from hepatic causes; 36%, from infections with or without GVHD; 15%, from pulmonary causes; and 18%, from other causes.

Overall, fewer nonablative than ablative patients had scores of 0 (29% versus 41%) and 1 to 2 (31% versus 35%), while more nonablative than ablative patients had scores of 3 or more (40% versus 24%).

Discussion

We previously reported on the importance of pretransplant comorbidities for outcomes after allogeneic HCT[18,19] using the CCI, given that it was a validated and simple scoring system for comorbidities. The studies were confined both by small sample sizes and the limited ability of the CCI to comprehensively capture comorbidities, particularly in patients given ablative conditioning. The current study refined comorbidity definitions, added newly identified comorbidities, and evaluated each comorbidity category by Cox regression hazard models. A new index of scores for each comorbidity was then developed and validated in another randomly selected group of HCT recipients. We found that the HCT-CI captured more pretransplant comorbidities and provided better assessment of NRM and survival risks compared with the original CCI.

The adverse impact of comorbidities on cancer patients was likely due to the physiologic burden of chronic disease and its interactions with cancer and cancer treatment.[42] Therefore, increased severity of comorbidities increased the risk of toxicities in response to specific treatments and, hence, shortened life expectancy and canceled gains derived from specific therapies.[43] There has been ample evidence in the literature that comorbidities and

Table 4. Definitions of comorbidities included in the HCT-CI and HCT-CI scores compared with original CCI scores

Comorbidity	Definitions of comorbidities included in the new HCT-CI	HCT-CI weighted scores	Original CCI scores*
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	1	0
Cardiac‡	Coronary artery disease,§ congestive heart failure, myocardial infarction, or EF ≤ 50%	1	1
Inflammatory bowel disease	Crohn disease or ulcerative colitis	1	0
Diabetes	Requiring treatment with insulin or oral hypoglycemics but not diet alone	1	1
Cerebrovascular disease	Transient ischemic attack or cerebrovascular accident	1	1
Psychiatric disturbance†	Depression or anxiety requiring psychiatric consult or treatment	1	Not included
Hepatic, mild‡	Chronic hepatitis, bilirubin > ULN to 1.5 × ULN, or AST/ALT > ULN to 2.5 × ULN	1	1
Obesity†	Patients with a body mass index > 35 kg/m^2	1	Not included
Infection†	Requiring continuation of antimicrobial treatment after day 0	1	Not included
Rheumatologic	SLE, RA, polymyositis, mixed CTD, or polymyalgia rheumatica	2	1
Peptic ulcer	Requiring treatment	2	1
Moderate/severe renal‡	Serum creatinine > 2 mg/dL, on dialysis, or prior renal transplantation	2	2
Moderate pulmonary‡	DLco and/or FEV$_1$ 66%-80% or dyspnea on slight activity	2	1
Prior solid tumor‡	Treated at any time point in the patient's past history, excluding nonmelanoma skin cancer	3	2
Heart valve disease	Except mitral valve prolapse	3	0
Severe pulmonary‡	DLco and/or FEV$_1$ ≤ 65% or dyspnea at rest or requiring oxygen	3	1
Moderate/severe hepatic‡	Liver cirrhosis, bilirubin > 1.5 × ULN, or AST/ALT > 2.5 × ULN	3	3

To convert creatinine from milligrams per deciliter to micromoles per liter, multiply milligrams per deciliter by 88.4.

EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide.

*Definitions of comorbidities included in the original CCI are defined in the appendix of a prior publication.[8]

†Newly investigated comorbidities.

‡Comorbidities with modified definitions compared with the original CCI.

§One or more vessel-coronary artery stenosis requiring medical treatment, stent, or bypass graft.

performance scales both independently predicted for outcome.[5] However, contrary to functional status, comorbidities presented the unique challenge of being a multidimensional variable, since diseases influencing mortality might not be the same as diseases influencing function or tolerance to treatment.[2] Translating comorbidities into weighted scores appeared to be the best way to use comorbidities for prediction of outcome.[2,8,44,45]

In the current study, we sought to modify the CCI in a way to improve sensitivity and specificity for predicting risks of NRM after HCT. The primary modification was refinement of several comorbidity definitions, which was done by introducing objective laboratory and functional testing data, thereby incorporating subclinical organ impairments that could result in partial compromise in patients subjected to the intensive physiologic challenge of HCT. Pulmonary function tests were used to define different grades of pulmonary comorbidities. Similarly, liver function tests and cardiac ejection fraction were added to definitions of hepatic and cardiac comorbidities. This introduction of laboratory data constituted the main change for increasing the sensitivity of the new HCT-CI, in particular since pulmonary and hepatic comorbidities achieved the highest prevalence among our transplant populations. The new HCT-CI captured 62% of patients with scores more than 0 compared with 12% captured by the original CCI. In particular, 59% of ablative patients had scores more than 0 using the HCT-CI compared with 10% by the original CCI.

The improvement in the new HCT-CI over the original CCI was demonstrated by likelihood ratio and *c* statistics. The improvement was likely related to the assignment of comorbidity weights specific to patients who underwent transplantation. Several common comorbidities were assigned higher weights than in the original CCI, likely reflecting their importance for tolerating HCT. In addition, new comorbidities, such as infections, obesity, and psychiatric disturbances, were found to be clinically relevant. The use of laboratory data in the new HCT-CI accounted for larger numbers of patients with scored comorbidities, which added to the utility of the new HCT-CI over the original CCI.

In all cases, the *c*-statistic calculated for the HCT-CI was 7 to 15 percentage points higher than that for the CCI, with the value for the CCI being only slightly better than a coin flip (0.50). Although the difference between HCT-CI and CCI was highly statistically significant, it should be noted that the magnitude of the *c*-statistic for the HCT-CI reflected a fairly modest level of prediction. This was not

Table 5. The new HCT-CI scores and prediction for NRM and survival the training versus the validation set

	Training set			Validation set		
		NRM			NRM	
Score	Patients, %	HR* (95% CI)	2-year, %	Patients, %	HR* (95% CI)	2-year, %
0	38	1	9	38	1	14
1	17	1.66 (0.9-3.1)	14	18	1.57 (0.7-3.3)	22
2	17	3.48 (2.0-6.0)	27	17	1.26 (0.6-2.8)	19
3	17	6.09 (3.7-10.1)	41	15	3.95 (2.1-7.5)	41
4 or more	11	6.93 (4.0-12.0)	43	13	3.05 (1.5-6.2)	40

For the training set, n = 708; for the validation set, n = 346.

*Adjusted for age, disease risk, and conditioning.

Table 6. HCT-CI versus CCI for prediction of 2-year NRM and survival in the validation set

	HCT-CI					CCI				
		NRM		Survival				NRM		Survival	
Score	No.	HR* (95% CI)	2-year, %	HR* (95% CI)	2-year, %	Score	No.	HR* (95% CI)	2-year, %	HR* (95% CI)	2-year, %
0	38	1.0	14	1.0	71	0	87	1.0	23	1.0	59
1 to 2	34	1.42 (0.8-2.7)	21	1.31 (0.8-2.0)	60	1	10	1.25 (0.6-2.5)	29	1.32 (0.8-2.2)	49
3 or more	28	3.54 (2.0-6.3)	41	2.69 (1.8-4.1)	34	≥ 2	3	1.46 (0.5-4.7)	25	2.78 (1.4-5.6)	17

*Adjusted for age, disease risk, and conditioning.

surprising given the multiple factors that also contributed to both NRM and survival and the smaller patient sample in the validation set.

Even though the HCT-CI scores were highly effective in predicting outcome of patients after HCT, other major pretransplant factors played significant roles. Age and disease stage of a specific hematologic malignancy were additional factors that should be considered when determining HCT risks. Age has been used independently to determine if patients should be referred for HCT and to stratify patients for the different intensity conditioning regimens.[46-49] For that reason and also since age is a demographic variable, we decided not to include age as a scored comorbidity in the HCT-CI. Instead, in the current study, age was used to adjust the Cox regression hazards' modeling for developing the new HCT-CI.

We and other investigators had shown the importance of single organ comorbidities and abnormal laboratory data, as markers of organ dysfunction, on outcome of patients who underwent transplantation.[50-52] However, none have attempted to evaluate multiple comorbidities into a scoring system with predictive power to determine survival risks after HCT. On the other hand, some disease-specific prognostic scoring systems[53,54] have been developed for assessing patient risk prior to specific therapies including HCT. However, none of those scores took into account the impact of different comorbidities on outcome.

There were several limitations to our study. The first was the retrospective nature of the data collection. Since we relied on data recorded in medical charts, some potentially important data might not have been recorded and hence not been included in this analysis. However, the introduction of laboratory and functional data, most of which were stored in the database, has likely reduced the possibility of missing comorbidities. To better address this problem, new protocols include prospective scoring of enrolled patients. The second limitation was that patients from the same institution were used to validate the new HCT-CI. Efforts are under way to further validate the HCT-CI on patients who underwent transplantation in other institutions. The third limitation was the lack of interrater and test-retest reliabilities in this study. However, reports on the original CCI had previously shown both good interrater reliability between 0.74 and 0.945 by interclass correlation coefficient in older cancer patients[5,55] and good test-retest reliability of 0.92.[56] Since we used many of the definitions of the CCI with additional refinements based on laboratory and functional testing, which were not subject to investigator variations, it was likely that the HCT-CI had at least the same interrater reliability as the original CCI. We plan to test the HCT-CI reliability among different investigators located in different institutions.

The current HCT-CI could be applied to a number of settings. Our study identified prevalent and prognostically important coexisting illnesses that should be measured and considered in clinical trials and HCT registries. This would help to extrapolate the results of transplant recipients with significant comorbidities to the entire spectrum of HCT patients. A second application of this HCT-CI involves the consideration of medical evidence for individual patients at the bedside. The index can be widely used by referring physicians, oncologists, and hematologists when assessing the risks of patients with hematologic diseases for specific therapies and particularly referral for HCT. Currently, we investigate the importance of the new HCT-CI on outcomes in disease-specific patients given HCT. The third application includes a wide area of research concerning the correlation between comorbidities captured by the HCT-CI and different complications after HCT, such as infections, GVHD, and respiratory or hepatic

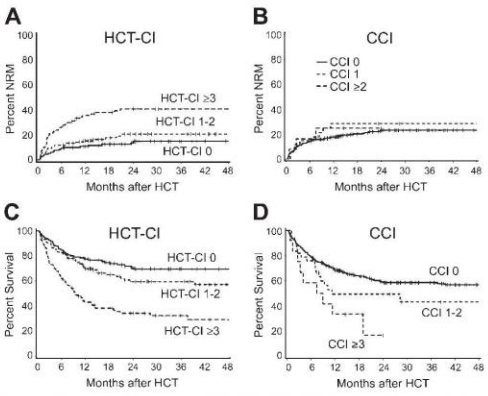

Figure 1. The HCT-CI compared with the CCI. Cumulative incidences of nonrelapse mortality (NRM) as stratified by the (A) new HCT-CI compared with (B) the original CCI, and Kaplan-Meier estimates of survival as stratified by (C) the new HCT-CI compared with (D) the original CCI among patients of the validation set. Only 13% of patients had scores of 1 or more when scored by the original CCI compared with 62% when scored by the new HCT-CI.

Table 7. Comparisons between the HCT-CI and the CCI in the validation set (n = 347) for events within first 2 years

	HCT-CI for 3 risk groups	CCI for 3 risk groups	*P**
Nonrelapse mortality			
c-statistic (SE*), overall	0.649 (0.029)	0.520 (0.019)	< .001
c-statistic (SE*), yes/no 2 y	0.685 (0.037)	0.532 (0.023)	< .001
c-statistic (SE*), yes/no 1 y	0.692 (0.036)	0.546 (0.025)	< .001
Likelihood ratio	26.6	1.2	
Adjusted likelihood ratio†	21.4	0.7	
Overall survival			
c-statistic (SE*), overall	0.624 (0.021)	0.536 (0.015)	< .001
c-statistic (SE*), yes/no 2 y	0.657 (0.031)	0.548 (0.019)	< .001
c-statistic (SE*), yes/no 1 y	0.661 (0.030)	0.561 (0.021)	< .001
Likelihood ratio	31.8	7.6	
Adjusted likelihood ratio†	23.7	7.1	

*Estimated from 100 bootstrap samples
†Adjusted for age, disease risk, and conditioning.

failure. The final application will be stratifying patients for eligibility of nonablative versus ablative HCT.

In summary, we have developed a new tool for capturing pretransplant comorbidities that could be used in predicting outcomes and stratifying patients for HCT. In the future, we will further validate this index on patients from different institutions and compare its performance to comorbidity indices other than the CCI, such as The Cumulative Illness Rating Scale and the Adult Comorbidity Evaluation (ACE-27). Further, it would be of interest to develop a scaling system for increments of age and different stages of diseases to be used in concert with the HCT-CI in assessing pretransplant patient risk for failure of disease-free survival.

Acknowledgments

The authors wish to thank Dr Karen Syrjala, associate professor for Psychiatry and Behavioral Sciences at University of Washington and FHCRC, for her valuable advice. We also thank the data coordinators, Chris Davis, Heather Hildebrant, and Deborah Bassuk, and the study nurses, Steve Minor, Mary Hinds, and John Sedgwick, for their invaluable help in making the study possible. Bonnie Larson, Helen Crawford, and Sue Carbonneau provided invaluable help with article preparation. We also wish to thank all the Transplant Teams.

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11.11 APPENDIX K CASE REPORT FORMS

Please enter this Data in Protocol Visit Transplant Note	ACUTE GVHD

Date of Visit:_____

Time point: ☐Day 100 ☐6 Mos ☐9Mos ☐12 Mos ☐18 Mos ☐24 Mos ☐36 Mos ☐48 Mos ☐Other _____

Does the patient have aGVHD _____Y/N

Type of aGVHD (Classic or Late Acute)_____

Late aGVHD type (Recurrent, Persistent, Late Onset)_____

aGVHD Organ involved (GI, Liver, Skin)	Date of Diagnosis	Biopsy Proven? (Y/N)	Date of Biopsy	Gluckbergs Organ Stage (0,1,2,3,4,unknown)	Overall Grade

Glucksberg Scoring System:

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

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 ++++	+++

Current Therapy (topical and systemic)	Current Dose	Date started	Date Ended	Response from Treatment (CR, PR, NR, PD)

PI Signature: _____ RN Signature: _____

Date of Visit: _____

Time point: □Day :100 □6 Mos □9Mos □12 Mos □18 Mos □24 Mos □36 Mos □48 Mos
□Other _____

Does the patient have cGVHD _____Y/N?

Type of cGVHD (Classic or Overlap)_____

Type of Onset (De Novo, Progressive, Quiescent)
_____

cGVHD Anatomic Site:	Grade	Date of Diagnosis:	Biopsy Proven? (Y/N)	Date of Biopsy:	Current Therapy (Topical and systemic)	Intensity of Immunosuppression □ None □ Mild (single agent prednisone < 0.5 mg/kg/day) □ Moderate (prednisone ≥ 0.5 mg/kg/day and/or any single agent/modality) □ High (2 or more agents/ modalities ± prednisone ≥ 0.5 mg/kg/day)

Global Stage (Mild, Moderate, Severe)_____

If 1-2 Grade 1=Mild If at least 1 Grade 3=severe Anything between=Moderate EXCEPT if Lung Grade 1=moderate Lung Grade 2=severe

Therapeutic intent at time of visit: Circle one

Does not require systemic therapy

Continue current systemic therapy

Alter systemic therapy due to toxicity

Decrease systemic therapy b/c cGVHD is better

Increase systemic therapy b/c cGVHD is stable or worse

Substitute systemic therapy due to lack of response

Withdraw systemic therapy due to lack of response

Clinician's Impression of Activity: Circle one

Active, irrespective of the level of current therapy

Highly active, irrespective of the level of current therapy

Inactive, off systemic therapy or immunosuppression

Inactive, on systemic therapy or immunosuppression

BOS Y/N_____

PI Signature: _____ RN Signature: _____

Please enter this Data in Transplant Note	Response Assessment

Date of Assessment: _____

Date of transplant: _____

Select Time point ☐Day 28 ☐Day 100 ☐3 Mos ☐6 Mos ☐9Mos ☐12 Mos

☐18 Mos ☐24 Mos ☐ 36 Mos ☐48 Mos ☐Other _____

A. Current Response Assessment: N/A N/E CR CRu PR SD PD Date_____

B. Minimal residual disease (circle one): Positive Negative Date_____

C. Central nervous system involvement (circle one): Yes No Unknown

Describe positive results below_____

D. Radiology response assessment:

Pet Scan Response Assessment: Y/N?_____If yes: CR/PR/SD/PD/NA/NE----------
CT Scan Response Assessment: Y/N?_____If yes: CR/PR/SD/PD/NA/NE----------
Other diagnostic studies_____

E. Pathology response assessment (complete for all performed evaluations):

- Bone Marrow Response Assessment: Positive Negative NE

  Morphology: Positive Negative Unknown

  a. Flow Cytometry: Positive Negative Unknown

  b. Molecular: Positive Negative Unknown

  c. Cytogenetics: Positive Negative Unknown

- LP Response Assessment: Positive Negative NE

Morphology: Positive Negative Unknown

a. Flow Cytometry: Positive Negative Unknown

b. Molecular: Positive Negative Unknown

c. Cytogenetics: Positive Negative Unknown

- Blood Response Assessment: Positive Negative NE

Morphology: Positive Negative Unknown

a. Flow Cytometry: Positive Negative Unknown

b. Molecular: Positive Negative Unknown

c. Cytogenetics: Positive Negative Unknown

- Tissue Response Assessment: Positive Negative NE

Morphology: Positive Negative Unknown

a. Flow Cytometry: Positive Negative Unknown

b. Molecular: Positive Negative Unknown

c. Cytogenetics: Positive Negative Unknown

- Other pathology studies response assessment (name study): _____

Positive Negative NE

PI Signature: _____Date_____

RN Signature: _____Date_____

132.