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A Randomized Phase II Study to Compare the Net Clinical Benefit of Cyclosporine and Sirolimus combined with MMF or Post-Transplant Cyclophosphamide as GVHD prophylaxis after HLA-Matched or HLA-Mismatched Unrelated G-CSF Mobilized Blood Cell Transplantation using Nonmyeloablative or Reduced Intensity Conditioning for Patients with Hematologic Malignancies

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1. Introduction

Hematopoietic cell transplantation (HCT) after nonmyeloablative (NMA) or reduced intensity conditioning (RIC) is effective therapy for patients with advanced hematologic malignancies who are considered to be at high risk for treatment related mortality (TRM) with high dose conditioning because of older age or comorbidities. Although such approaches have decreased early mortality and have expanded transplant as a therapeutic option for a larger number of patients, graft-vs-host-disease (GVHD) remains a major cause of morbidity and mortality. Particularly in HLA-mismatched transplants, while NMA and RIC approaches has been shown to establish stable donor engraftment, rates of acute and chronic GVHD and nonrelapse mortality (NRM) remain high (1).

Current standard GVHD prophylaxis after nonmyeloablative conditioning at our center consists of a calcineurin inhibitor [cyclosporine (CSP) or tacrolimus] and mycophenolate mofetil (MMF) (2, 3). Recent studies have investigated the benefit of adding sirolimus to this standard regimen. The results of Protocol 1938, a randomized Phase II study comparing 3 different immunosuppression regimens, showed that the addition of sirolimus to tacrolimus and MMF after matched unrelated donor transplantation using nonmyeloablative conditioning reduced the incidence of grade II-IV acute GVHD compared to tacrolimus and MMF only (64%, vs. 47%, arm 1 vs. 3 hazard ratio 0.62; p=0.04) (4). Cumulative incidence of chronic GVHD at 2 years was similar (60% vs. 59%, HR 1.10; p=0.67). Protocol 2206 evaluated the use of the combination of sirolimus, MMF, and tacrolimus after HLA class I or II-mismatched nonmyeloablative transplants. To date, 55 patients have been enrolled. The cumulative incidence of Grade II and III acute GVHD in 42 patients was 42% and 2% respectively at 120 days. No Grade 4 acute GVHD has been reported.

Based on these promising results, a multi-center randomized phase III trial (Protocol 2448) was conducted comparing the effectiveness of CSP/MMF with or without sirolimus in preventing grades II-IV acute GVHD after matched unrelated nonmyeloablative HCT. Both arms used CSP until day 96 with taper by day 150. In Arm 1, MMF was given tid until day 30, then bid until day 150 with a taper through day 180. In Arm 2 MMF was given through day 40, and stopped without taper, and sirolimus was given until day 150 with a taper though day 180. At planned interim analysis, 158 patients were enrolled with median follow-up of 24 months. The trial was closed due to significantly lower day 100 cumulative incidence of acute GVHD (grades II-IV: 53% vs. 25%, p=0.0001; grades III-IV: 8% vs. 2%, p=0.04) and 1-year cumulative incidence of non-relapse mortality (NRM) (15 vs. 5%, p=0.007) in the triple therapy arm. The 1-year cumulative incidence of chronic extensive GVHD, relapse/progression, and overall survival were similar.

Thus the addition of sirolimus to MMF and cyclosporine significantly reduced the risk of Grades II-IV and III-IV acute GVHD and NRM with no increased risk of relapse or progressive malignancy. However, rates of chronic GVHD were similar to prior studies (49% and 48% in Arms 1 and 2, respectively). Recent studies investigating the use of post-transplant high-dose cyclophosphamide (PTCy) given early after HLA-haploidentical or matched bone marrow as well as mobilized blood transplant have demonstrated low rates of chronic GVHD of 5-16% (5-8). Thus, we now propose to combine a sirolimus-based GVHD prophylaxis regimen with PTCy to evaluate whether the rate of chronic GVHD can be improved while maintaining the favorable effect on acute GHVD. As calcineurin inhibitors are an important backbone of GVHD prophylaxis and because prolonged course of MMF does not appear to decrease the incidence of GVHD among unrelated PBSC recipients after nonmyeloablative conditioning (2, 4), we will replace MMF with PTCy. We propose a two-arm, Phase II study for recipients of matched unrelated and mismatched (MHC class I or II) transplants with randomization to a GVHD prophylaxis regimen consisting of cyclosporine/sirolimus combined with either MMF or PTCy.

2. Background

FHCC Protocol 1938

The goal of Protocol 1938 (4) was to evaluate in a phase II randomized trial, which of three immunosuppression drug combinations was most promising in preventing acute GVHD following nonmyeloablative conditioning consisting of fludarabine 30mg/m^2 on days -4 to -2 and 2-3Gy TBI and matched unrelated donor transplant. Each of the combinations used MMF every 8 hours through day 30 and then MMF was given every 12 hours. The duration of MMF administration varied between the three arms. In each arm CSP was substituted for tacrolimus based on the previously observed lower incidence of acute GVHD when MTX was combined with tacrolimus instead of CSP after high-dose allogeneic HCT (9). However, this finding did not hold out in subsequent analysis including the patients from the other centers that participated in the trial (personal communication, Paul Martin). Patients were randomized into three arms: Arm 1 – tacrolimus 180 days and MMF 95 days (n=69); Arm 2 – tacrolimus 150 days and MMF 180 days (n=71); Arm 3 – tacrolimus 150 days, MMF 180 days, and sirolimus 80 days (n=68). Sustained engraftment was seen in all patients. Grade II-IV acute GVHD in the 3 arms were 64%, 48%, and 47% at Day 150 (Arm 3 vs. Arm 1 hazard ratio 0.62; p=0.04). Corresponding with this reduction in acute GVHD, systemic steroid use was lowest in Arm 3 (32% vs. 55% in Arm 1 and 49% in Arm 2, overall p=0.009 by hazard ratio analysis). Furthermore, the incidence of CMV reactivation at Day 150 was lowest in Arm 3 (arm 1, 54%; arm 2, 47%; arm 3, 22%; overall P=0.002 by hazard ratio analysis). In the analysis of the impact of sirolimus on the individual grades of acute GVHD, the benefit was only observed for grade II. No difference between treatment arms was observed for grade III-IV acute GVHD, chronic GVHD, non-relapse mortality and relapse/progression. When the results of protocol 1938 were compared to a historical cohort of 174 unrelated nonmyeloablative transplants treated with a combination of CSP and MMF, no difference was observed in the cumulative incidences of nonrelapse mortality, relapse/progression, and acute and chronic GVHD as compared to the tacrolimus/MMF only arms, suggesting that the substitution of CSP for tacrolimus does not entail additional GVHD control.

FHCC Protocol 2206

Protocol 2206 was a Phase II study designed to evaluate the efficacy of triple therapy using sirolimus, MMF and cyclosporine after mismatched donor transplant following nonmyeloablative conditioning consisting of fludarabine 30mg/m^2 on days -4 to -2 and 2-3Gy TBI. MMF was given tid day 0 to +30 and bid to Day +100 and tapered to day +150. Cyclosporine 5 mg/kg po bid was given Day -3 to 150 then taper to day +180, and sirolimus was given 2mg po daily starting Day -3 to +180 and tapered to day +365. Fifty-five subjects have been enrolled as of March 2016. Interim analysis showed cumulative incidence of Grade II and III acute GHVD at 42% and 2% at 120 days, respectively. No Grade IV acute GVHD has been reported. At 2 years, the cumulative incidence of chronic GVHD was 59%. Non-relapse mortality at 2 years was 19% and overall survival was 66%.

FHCC Protocol 2448

Given the promising result of reduction in acute GVHD seen with the addition of sirolimus to the standard regimen using calcineurin-inhibitor and MMF, the goal of the randomized Phase III study, Protocol 2448, was to compare the effectiveness of the 2 GVHD prophylaxis regimens in preventing acute grades II-IV acute GVHD after nonmyeloablative matched unrelated HCT. Both arms used CSP until day 96 then tapered off by day 150. In Arm 1 (similar to the protocol 1938 Arm 2 above), MMF was given tid until day 30, then bid until day 150 with a taper through day 180. In Arm 2 MMF was given through day 40, and then discontinued without a taper and sirolimus was given until day 150 with a taper though day 180. The primary objective was to compare the respective incidences of grades II-IV acute GVHD. At planned interim analysis, 158 patients were enrolled and the trial was closed due to significantly lower risks of

grades II-IV and III-IV acute GVHD and non-relapse mortality in the triple therapy arm. At a median follow-up of surviving patients of 24 months, the day 100 cumulative incidence of grades II-IV acute GVHD and grades III-IV acute GVHD were significantly lower in Arm 2 (53% vs. 25%, p=0.0001 and 8% vs. 2%, p=0.04, respectively). The 1-year cumulative incidence of non-relapse mortality was lower in Arm 2 (15% vs. 5%; p=0.007). However, the 1-year cumulative incidence of chronic extensive GVHD was similar in Arm 1 vs. Arm 2 (49% and 48%, p=0.94) and relapse/progression was similar at 1 year (21% vs. 19%, p=0.86). Overall survival and progression-free survival at 1 year for Arm 1 vs. Arm 2 were 72% vs. 85% (p=0.03) and 65% vs. 77% (p=0.08), respectively.

D. Sirolimus

1. Mechanism of action.

i. Immunomodulatory effect. Sirolimus was isolated in a discovery program for novel antifungal agents. It is a macrocyclic lactone fermentation product of Streptomyces hygroscopicus, an actinomycete that was isolated from a soil sample collected from Rapa Nui (Easter Island). Although, the activity of sirolimus depends on its binding to the same class of cytosolic binding proteins (immunophilins) as CSP and tacrolimus, its mechanism of action is unique. The complex of CSP or tacrolimus with their respective immunophilins inhibit calcineurin, which in turn impairs signaling through the T-cell receptor, reducing the expression of cytokines important for the antigen specific expansion of T-cells (e.g. IL-2, IL-3, IL4 and TNFα.), hereby arresting their cell cycle in G_0 to G_1. Sirolimus has no effect on the calcineurin pathway, but inhibits the mammalian target of rapamycin (mTOR) protein kinase, which promotes cell proliferation and is a key regulatory kinase in cell cycle control. In contrast to CSP and tacrolimus inhibition of T-cell receptor induced activation and cytokine secretion, the sirolimus-immunophilin complex inhibits the T-cell's response to cytokines, hereby arresting the cell cycle at a later stage (G_1 to S phase) (10). Although the mechanism is not fully understood, mTOR inhibition has the ability to promote antigen specific expansion of regulatory T-cells (T_{reg}) and skew the $CD4^+$ phenotype towards the tolerance inducing $CD4^+CD25^{high}$ (11, 12). mTOR inhibition mainly blocks signaling pathways important for the expansion of T effector cells, while IL-2 dependent JAK/STAT signaling which is important for T_{reg} proliferation is unaffected (13, 14). The preferential expansion of T_{reg} is attenuated when sirolimus is used in combination with CSP (12). In a murine bone marrow transplantation model transfer of T_{reg} could prevent GVHD induced by non-regulatory T-cells, without interfering with engraftment or the graft versus leukemia effect (15-17).

Another immunomodulatory property of sirolimus is its ability to inhibit dendritic cell activity. The mTOR pathway has been demonstrated to be important for the in vitro development of CD34-derived dendritic cells, with inhibition by sirolimus reducing antigen uptake, lipopolysaccharide induced cytokine secretion, CCR7 expression and T-cell stimulation (18).

ii. Viral amplification. Inhibition of mTOR may also have effects on viral amplification, as CMV specifically upregulates the mTOR pathway during replication (19). In allogeneic HCT and solid organ transplantation lower risk of CMV activation has been reported in patients treated with sirolimus (20, 21).

iii. Antineoplastic effects. The mTOR signaling pathway is often constitutively activated in various human cancers. Sirolimus has been shown to induce cell cycle arrest *in-vitro* in both B-CLL and diffuse large B-cell lymphoma cells, and its efficacy as an antiangiogenic agent has been demonstrated in several experimental cancer models (22-26). In the context of solid organ transplantation, a retrospective analysis of transplant registry data from 33249 recipients of necro-kidney allografts, showed a decreased risk of developing any *de novo* cancer in recipients treated with mTOR based immunosuppression (sirolimus or an analog), as compared to recipients treated with non-mTOR inhibitor based immunosuppression (hazard ratio: 0.39; 95% CI: 0.24-0.64; P=0.0002) (27).

2. Sirolimus for acute GVHD prophylaxis.
Four clinical trials with sirolimus have been published from the Dana-Farber Cancer Institute (21, 28-30). In all 4 trials similar GVHD prevention with sirolimus in combination with tacrolimus ± abbreviated MTX dosing (5 mg/m^2 given every other day starting on day +1 for 3 to 4 days) was used. Sirolimus was started at day -3 with an oral loading dose of 12 mg, and followed by a single daily dose of 4 mg, with a target serum concentration of 3-12 ng/ml. Tacrolimus was administrated at 0.02 – 0.05 mg/kg/day intravenously by continuous infusion beginning on day-3 with a target serum concentration of 5-10 ng/ml. Control of GVHD was excellent independently of conditioning regimen (myeloablative vs reduced intensity conditioning (fludarabine 120 mg/m^2 and busulfan 3.2 mg/kg)), donor source (related vs unrelated) and stem cell source (bone marrow vs. peripheral blood stem cells) (Table 1).

In a retrospective study by Armand et al. (31) 190 patients undergoing transplantation for lymphoma were analyzed. The cohort consisted of patients treated with myeloablative (n=64) or reduced intensity conditioning (n=126) and transplanted with bone marrow (n=15), PBSC (n=168) or umbilical cord (n=7) grafts from either related (n=78) or unrelated donors (n=112). One-hundred-and-twenty-six of the patients were treated with a combination of sirolimus with tacrolimus ± MTX, similar to the above mentioned Dana-Farber trials, while the remaining 90 patients received calcineurin inhibitor and MTX based immunosuppression. Although overall survival was superior in patients treated with sirolimus when the whole cohort was analyzed, multivariate analyses revealed that the benefit was restricted to patients undergoing reduced intensity conditioning (sirolimus group (n=103) 66% vs non-sirolimus (n=23) 38%; p=0.007) independently of MTX. In the same subgroup of patients sirolimus treatment was also associated with lower probability of progression (42% vs 74%, p=0.001), while no effect was observed on NRM (sirolimus group 14% vs non-sirolimus group 9%, p=0.6). No statistically significant associations between sirolimus and the incidences of GVHD were observed (sirolimus-group vs non-sirolimus: acute GVHD: grade II-IV 14% vs 22%, p=0.6; grade III-IV 6% vs 13%, p=0.4, chronic GVHD 63% vs 48%, p=0.2).

In a recent multicenter Phase III trial (32), the combination of tacrolimus, sirolimus started on day -3, and low-dose methotrexate (5mg/m^2 days 1, 3, 6) was compared to standard regimens (tacrolimus and low-dose methotrexate or cycloporine and MMF) in adults undergoing RIC HCT for lymphoma. 139 patients were randomized. While there was no difference in survival, PFS, relapse, NRM, or chronic GVHD at 2 years, the sirolimus group had significantly lower incidence of grade II-IV acute GVHD (9% vs. 25%, p=0.015).

Table 1. Summary of clinical trials

	Antin et al.(28)	Cutler et al.(21)	Cutler et al.(29)	Alyea et al.(30)	FHCC protocol 1938
Sample size	41	30	83	91	62
Median age, yrs (range)	42 (19-62)	42 (19-54)	42 (18-59)* 44 (22-54)**	57 (20-69)	60 (13-75)
HLA match					
HLA matched, related		30 (1 HLA matched parent)	53	46 (1 HLA-C MM)	
HLA matched, unrelated	29		30	45 (7 HLA-C MM)	62
Hematopoietic cell source	BM	PBSC	PBSC	PBSC	PBSC
Conditioning	MAC	MAC	MAC	RIC	NMA
Immunosuppression					
Sirolimus, daily dose (mg/kg) (start day/start taper/end)	4 (-3/+63/+182)	4 (-3/+100/+182)	4 (-3/+100/+182)	4 (-3/NA/NA)	2 (-3/NA/+80)
Tacrolimus, daily dose (mg/kg) (start day/start taper/end)	0.02 (-3/+63/+182)	0.02 (-3/+100/+182)	0.02 (-3/+100/+182)	0.05 (-3/NA/NA)	0.12 (-3/+100/+150)
MMF, dose (mg/kg) (start day/start taper/end)					0.45/0.30 (-3/+100/+180)
Other	MTX			MTX	
GVHD (%)					
Acute grade. II-IV, %	26	10 (only gr. II)	21	10	45
Acute grade. III-IV, %	13	0	NA	NA	11
Chronic, %	44	11 out of 28 patients	59	40	46
Survival					
treatment-related mortality, %	15 (day 100)	6 (1 yr)	5 (day 100)	6 (2 yrs)	3 (day 200)
1 year relapse-free survival, %	46	71	72	47	NA
1 year overall survival, %	51	67	77	74	47 (2 yrs)

BM, bone marrow; PBSC, peripheral blood stem cells; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; NMA, non-myeloablative conditioning; MMF, mycophenolate mofetil ; MTX, methotrexate; GVHD, graft versus host disease; NA, not available; *, patients transplanted with matched related donors; **, patients transplanted with matched unrelated donors.

E. The use of 3 Gy TBI in place of 2 Gy TBI for patients at higher risk of rejection or higher risk of relapse:

We have shown that the risk of rejection increases for certain diseases, as well as for those patients previously transplanted with either syngeneic or allogeneic stem cells. Please see section 11.H (Conditioning Regimen) for criteria for 3 Gy TBI. Those at higher risk of disease relapse or progression will also be eligible for 3 Gy TBI.

F. Use of Post-Transplant Cyclophosphamide for GVHD prophylaxis after nonmyeloablative HCT

Using CY after HCT

High doses of CY are not myeloablative, but are immunosuppressive due to its unique pharmacology. Jones (33) and Kastan (34) amongst others have correlated high levels of aldehyde dehydrogenase (found in hematopoietic progenitor cells) to CY resistance and low levels (found in T and B lymphocytes, NK cells) to CY sensitivity. Immature erythroid precursors express aldehyde dehydrogenase in intermediate levels. Storb and colleagues (35) were the first to show that Rhesus monkeys receiving up to 200 mg/kg CY will have full hematological recovery without need for marrow infusion. Brodsky et al (36) showed similar results in the clinical setting, where doses of CY 200 mg/kg can be used to treat severe autoimmune diseases without impacting hematological recovery or

needing autologous stem cell rescue. In the MHC-mismatched murine model, Luznik and colleagues (37) confirmed CY's ability to selectively deplete alloreactive, proliferating host and donor lymphocyte clones after using the FLU/TBI-based nonmyeloablative conditioning regimen developed at FHCC. However, it could not be tested in these studies whether the beneficial effect of ablating these proliferating T cells to decrease the incidence of GVHD would have any undue effects in a malignant disease model. Further, work done at the NIH (38) has shown that in a fully MHC-mismatched murine transplant model, FLU and CY are synergistic and can successfully and preferentially deplete host T cells when compared to myeloablative doses of TBI.

Results of phase II nonmyeloablative clinical trials using HLA-haploidentical donors: the Johns Hopkins University (JHU) and Seattle collaborative experience

Based on a successful Phase I study conducted by O'Donnell and colleagues at JHU (n=13) (39), a Phase II

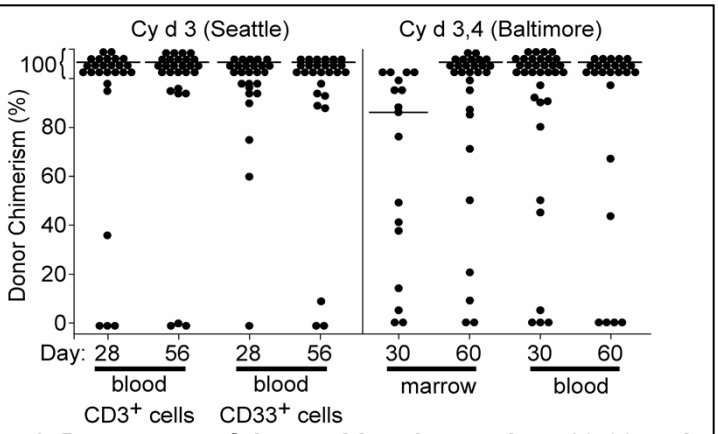


Figure 1. Percentage of donor chimerism at days 28-30 and 56-60 by group.

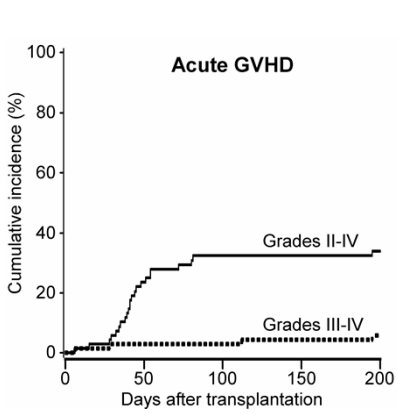
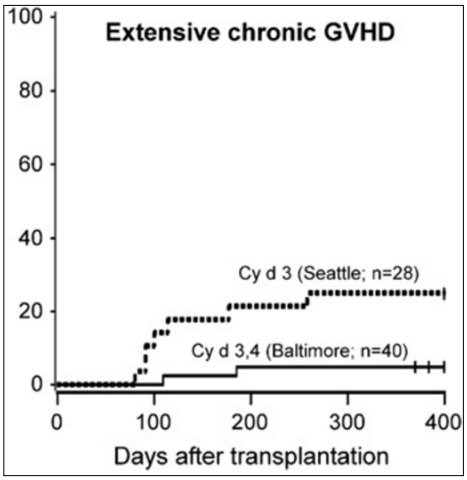


Figure 2. Cumulative incidence of acute GVHD in both groups.

study was developed. Between 1999 and 2006, 68 patients with high-risk hematological malignancies requiring allogeneic HCT but lacking HLA-matched donors were enrolled on 2 nonmyeloablative, HLA-haploidentical Phase II clinical trials in Seattle and at Johns Hopkins University in Baltimore (5). These studies differed by immunosuppression only: the Baltimore regimen utilized 2 doses while the Seattle regimen used 1 dose of CY after HCT. 25% of all patients were from ethnic minorities, in part due to the greater genetic diversity seen in African Americans, and highlighting the lower prevalence of underrepresented populations in bone marrow registries. Conditioning consisted of CY 14.5 mg/kg/day (days -6 to -5), FLU 30 mg/kg/day (days -6 to -2), and 2 Gy TBI, followed by unmanipulated, HLA-haploidentical marrow allografts, with post-grafting immunosuppression consisting of CY 50 mg/kg (day +3 ± 4), MMF, and tacrolimus (both beginning 24 hours after the last dose of CY). Because of the use of HLA-haploidentical grafts, strong immunological barrier needed to be overcome with this regimen. Rejection occurred on average in 9 of 66 (13%) evaluable patients. Most patients converted to full donor chimerism by day +60 (Figure 1). More notable was the cumulative incidence of grade III GVHD of 6% at 1 year with no grade IV GVHD (Figure 2). NRM was 4% at day +100 and 15% at 1 year with infections being the leading causes of NRM. However, relapse was the primary contributor to low overall survival, with 58% of patients relapsing by 2 years after transplantation, leading to an overall survival of 36% by 2 years. The risk of chronic GVHD appeared to be lower in patients who received CY 50 mg/kg on days 3 and 4 after HCT, compared to those who received CY 50 mg/kg only on day 3 alone (Figure 3).

Figure 3



While PTCy has been investigated in the context of haploidentical donors with bone marrow as the source, recent studies have reported use of PTCy after HLA-matched mobilized blood transplants with similarly low rates of chronic GVHD after RIC or myeloablative conditioning (8, 40, 41). Protocol 2541 investigated the use of PTCy after G-CSF-mobilized blood cell transplantation in matched related and unrelated donors after myeloablative conditioning consisting of fludarabine and targeted busulfan or TBI. PTCy was given at 50 mg/kg on day 3 and 4 after transplantation, and cyclosporine was started on day 5. The cumulative incidence of grade II-IV acute GVHD was 77% with no grade 3-4 acute GVHD. The cumulative incidence of NIH-defined chronic GVHD requiring systemic immunosuppression at 1 year was 16% (95% confidence interval [CI], 5-28%) (8).

Post-Transplant Cyclophosphamide in combination with sirolimus

Recently, PTCy has been combined with sirolimus as a calcineurin inhibitor-free approach to GVHD prophylaxis after HLA-matched peripheral blood HCT with the goal of reducing acute GVHD and TRM (42-44). Greco et al. (43) recently reported on the use of post-grafting immunosuppression with PTCy (50 mg/kg) on days 3 and 4 followed by sirolimus maintained until 3-6 months after transplant after myeloablative conditioning consisting of treosulfan, fludarabine, and melphalan and HLA-matched G-CSF mobilized peripheral blood stem cell transplant in 28 patients. MMF was added for recipients of matched unrelated donor transplants (n=13) and withdrawn at day 30. The cumulative incidence of grades II-IV and III-IV acute GVHD at 100 days was 23% and 4%, respectively, and the cumulative incidence of chronic GVHD was 13% at 1 year. With median follow-up of 225 days (56-841 days) for surviving subjects, the 2 year estimated survival was 64% and composite end point of GRFS was 45% at 1 year (43). Solomon et al. reported on the use of PTCy given on days 3 and 4 and sirolimus starting on day 5 after reduced-intensity conditioning regimen of fludarabine, busulfan, and cyclophosphadmie followed by HLA-matched related or unrelated mobilized peripheral blood transplant (44). Sirolimus was discontinued without taper at day 90-100 in the absence of GVHD. The cumulative incidence of grade II-IV and III-IV acute GVHD was 46% and 15%, respectively, and cumulative incidence of extensive chronic GVHD was 31% (44). Notably, MMF was not included in this regimen and no graft rejection was reported.

The combination of sirolimus, cyclosporine, and PTCy has not been previously studied, although available data on the combination of sirolimus with PTCy does not indicate the presence of unexpected toxicities. In the study by Solomon et al. (44) four patients were switched from sirolimus to tacrolimus due to cytopenia (n=2) or reversible and non-fatal hepatic sinusoidal obstruction syndrome (n=2); however, sirolimus trough levels were ≥10 ng/mL in 3 of the 4 patients.

3. Proposal

We hypothesize that combining a sirolimus-based GVHD regimen with PTCy will decrease chronic GVHD while preserving favorable effect on acute GVHD. We propose a randomized phase II trial to compare the effectiveness of cyclosporine and sirolimus combined with either MMF or PTCy as GVHD prophylaxis after nonmyeloablative or reduced-intensity conditioning and HLA-matched or HLA-mismatched (MHC class I or II) unrelated G-CSF mobilized blood cell transplant. Randomization is based upon transplant center (FHCC vs other) and donor HLA-match (HLA-matched or mismatched as defined below).

4. Primary Objective

Compare chronic GVHD-free and relapse-free survival (CRFS) after transplant between the 2 GVHD prophylaxis regimens. CRFS is a time to event outcome defined as moderate to severe chronic GVHD based on the NIH consensus criteria, disease relapse, or death by any cause.

5. Secondary Objectives

Compare rates of acute (grades II-IV and III-IV) and moderate and severe chronic GVHD (based on NIH consensus criteria), relapse, non-relapse mortality, progression or relapse-free survival, and overall survival between the 2 regimens.

6. Patient Selection

A. Inclusions

Ages >50 years with hematologic malignancies treatable by unrelated HCT.

Ages 18 to 50 years with hematologic diseases treatable by allogeneic HCT who through pre-existing medical conditions or prior therapy are considered to be at high risk for regimen related toxicity associated with a high dose transplant.

Ages 18 to 50 years with chronic lymphocytic leukemia (CLL).

Ages 18 to 50 years with hematologic diseases treatable by allogeneic HCT who refuse a high-dose HCT. Transplants must be approved for these inclusion criteria by the principal investigator.

The following diseases will be permitted although other diagnoses can be considered if approved by PCC and the principal investigator.

- **Aggressive nonHodgkin lymphomas (NHL) and Other Histologies Such as Diffuse large B cell NHL**– not eligible for autologous HCT, not eligible for high-dose allogeneic HCT, or after failed autologous HCT.
- **Mantle Cell NHL** -may be treated in first CR. (Diagnostic LP required pre-transplant)
- **Low grade NHL**– with < 6 month duration of CR between courses of conventional therapy
- **CLL** – must have either 1) failed to meet NCI Working Group criteria for complete or partial response after therapy with a regimen containing FLU (or another nucleoside analog, e.g. 2-CDA, pentostatin) or experience disease relapse within 12 months after completing therapy with a regimen containing FLU (or another nucleoside analog); 2) failed FLU-CY-Rituximab (FCR) combination chemotherapy at any time point; or 3) have "17p deletion" cytogenetic abnormality. Patients should have received induction chemotherapy but could be transplanted in 1st CR; or 4) Patients with a diagnosis of CLL (or small lymphocytic lymphoma) or diagnosis of CLL that progresses to prolymphocytic leukemia (PLL), or T-cell CLL or PLL. 5) Patients failing to achieve a response to ibrutinib as first-line therapy; 6) Patients not

responding to ibrutinib, idelalisib, or venetoclax as salvage therapy or intolerant of these agents as salvage therapy due to side effects.

- **Hodgkin Lymphoma** – must have received and failed frontline therapy.
- **Multiple Myeloma** – must have received prior chemotherapy. Consolidation of chemotherapy by autografting prior to nonmyeloablative HCT is permitted.
- **Acute Myeloid Leukemia (AML)** – must have < 5% marrow blasts at the time of transplant.
- **Acute Lymphocytic Leukemia (ALL)** – must have <5% marrow blasts at the time of transplant.
- **Chronic Myeloid Leukemia (CML)** – Patients in CP1 must have failed or be intolerant of TKIs. Patients beyond CP1 will be accepted if they have <5% marrow blasts at time of transplant.
- **Myelodysplasia (MDS)/Myeloproliferative Syndrome (MPS)/CMML** – Patients must have <5% marrow blasts at time of transplant.
- **Waldenstrom's Macroglobulinemia –** must have failed 2 courses of therapy.
- **Mixed Phenotype Acute Leukemia (MPAL)** – must have < 5% marrow blasts at the time of transplant.
- **Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) – must be in complete remission at the time of transplant**

B. Exclusions:

1. Patients with rapidly progressive intermediate or high grade NHL.
2. Patients with a diagnosis of CMML who have not received induction chemotherapy.
3. Patients with MDS-EB or AML who have not received myelosuppressive chemotherapy i.e. induction chemotherapy or at least 4 cycles of a venetoclax-containing regimen will be excluded from Regimen B conditioning (Fludarabine and TBI).
4. CNS involvement with disease refractory to intrathecal chemotherapy. For LP requirement, see Standard Practice Guidelines.
5. Presence of circulating blasts determined to be associated with disease (in the blood) for patients with AML, ALL or CML.
6. Presence of ≥ 5% circulating leukemic blasts (in the blood) detected by standard pathology for patients with MDS/MPS/CMML
7. Fertile men or women unwilling to use contraceptive techniques during and for 12 months following treatment.
8. Females who are pregnant or breast-feeding.
9. Patients with active non-hematological malignancies (except non-melanoma skin cancers) or those with non-hematological malignancies (except non-melanoma skin cancers) who have been rendered with no evidence of disease, but have a greater than 20% chance of having disease recurrence within 5 years.
   This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy.
10. Fungal infections with radiological progression after receipt of amphotericin B or active triazole for greater than 1 month.
11. Organ dysfunction.
    a. Cardiac ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of < 26%). Ejection fraction is required if age > 50 years or there is a history of

anthracycline exposure or history of cardiac disease. Patients with a shortening fraction < 26% may be enrolled if approved by a cardiologist.

b. Pulmonary:
   i) DLCO < 40%, FEV1 <40% and/or receiving supplementary continuous oxygen. When PFTs cannot be obtained, the 6-minute walk test (6MWT, also known as exercise oximetry) will be used: Any patient with oxygen saturation on room air of <89% during a 6MWT will be excluded.
   ii) The FHCC PI of the study must approve of enrollment of all patients with pulmonary nodules.

c. Liver function abnormalities: Patients with clinical or laboratory evidence of liver disease would be evaluated for the cause of liver disease, its clinical severity in terms of liver function, and the degree of portal hypertension. Patients will be excluded if they are found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bridging fibrosis, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3 mg/dL, or symptomatic biliary disease.

12. Karnofsky scores < 60 (see appendix B)
13. Patient has poorly controlled hypertension and on multiple antihypertensives
14. HIV positive patients.
15. Active bacterial or fungal infections unresponsive to medical therapy.
16. The addition of cytotoxic agents for "cytoreduction" with the exception of tyrosine kinase inhibitors (such as imatinib), cytokine therapy, hydroxyurea, low dose cytarabine, chlorambucil, or rituxan will not be allowed within three weeks of the initiation of conditioning.
17. Patients on hemodialysis.

7. Donor Selection

A. Inclusions – HLA-Matched Unrelated Donor

1. **FHCC matching allowed will be Grades 1.0 to 2.1 (See Standard Practice Guidelines)**: Unrelated donors who are prospectively:
   i) Matched for HLA-A, B, C, DRB1 and DQB1 by high resolution typing;
   ii) ***Only a single allele disparity*** will be allowed for HLA-A, B, or C as defined by high resolution typing (see **Standard Practice Guidelines for other donor selection details**).
2. Donors are excluded when preexisting immunoreactivity is identified that would jeopardize donor hematopoietic cell engraftment. This determination is based on the standard practice of the individual institution. The recommended procedure for patients with 10 of 10 HLA allele level (phenotypic) match is to obtain a panel reactive antibody (PRA) screens to class I and class II antigens for all patients before HCT. If the PRA shows >10% activity, then flow cytometric or B and T cell cytotoxic cross matches should be obtained. **The donor should be excluded if any of the cytotoxic cross match assays are positive**. For those patients with an HLA Class I allele mismatch, flow cytometric or B and T cell cytotoxic cross matches should be obtained regardless of the PRA results. A positive anti-donor cytotoxic crossmatch is an absolute donor exclusion.
3. Patient and donor pairs homozygous at a mismatched allele in the graft rejection vector are considered a two-allele mismatch, i.e., the patient is A*0101 and the donor is A*0102, and **this type of mismatch is not allowed.**
4. Only G-CSF mobilized PBSC will be permitted as a HSC source on this protocol.

B. Inclusions – HLA-Mismatched Unrelated Donor

1. Unrelated volunteer donors who are mismatched with the recipient within one of the following limitations:
    a) Mismatch for one HLA class I antigen with or without an additional mismatch for one HLA-class I allele, but matched for HLA-DRB1 and HLA-DQ, OR
    b) Mismatched for two HLA class I alleles, but matched for HLA-DRB1 and HLA-DQ
    c) HLA class I HLA-A, -B, -C allele matched donors allowing for any one or two DRB1 and/or DQB1 antigen/allele mismatch
2. HLA-matching must be based on results of high resolution typing at HLA-A, –B, -C, - DRB1, and –DQ.
3. If the patient is homozygous at the mismatch HLA class I locus or II locus, the donor must be heterozygous at that locus and one allele must match the patient (i.e., patient is homozygous A*01:01 and donor is heterozygous A*01:01, A*02:01). This mismatch will be considered a one-antigen mismatch for rejection only.

C. Exclusions

1. Donor (or centers) who will exclusively donate marrow.
2. Donors who are HIV-positive and/or, medical conditions that would result in increased risk for G-CSF mobilization and harvest of PBSC.
3. Patients who are homozygous at the mismatched HLA class I or II locus, the donor is excluded if homozygous at the mismatched locus (i.e., patient is homozygous A *01:01 and donor is homozygous A *02:01); this type of mismatch is considered a two-antigen mismatch and is not allowed.

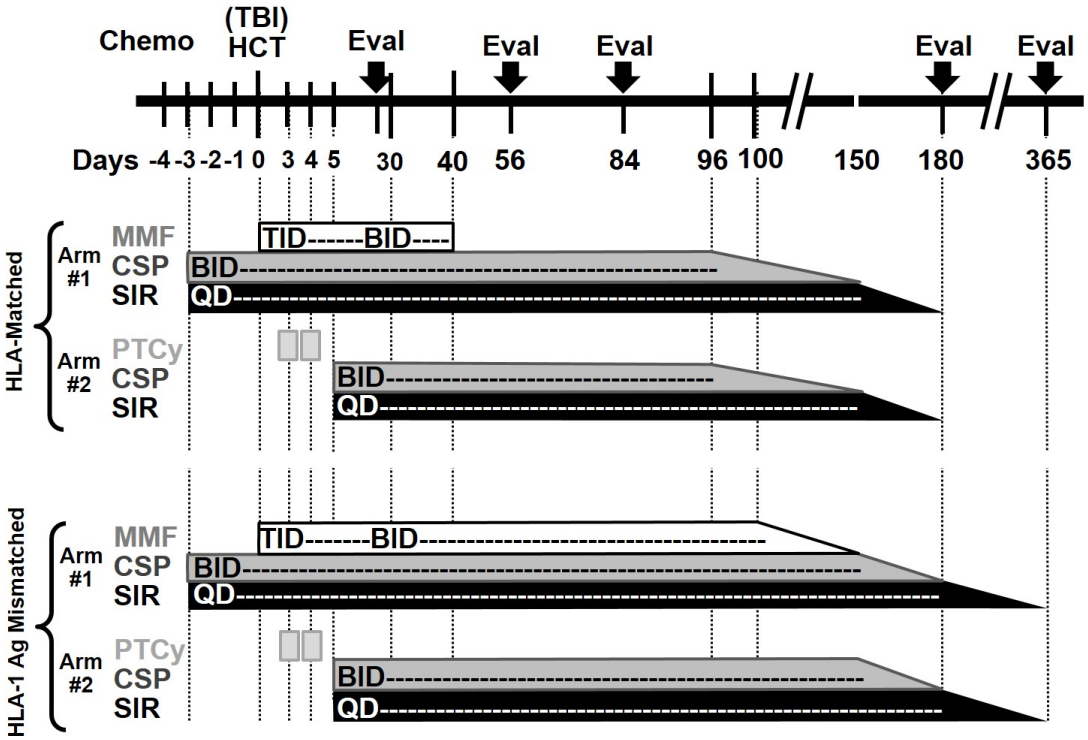
8. Informed Consent

A conference will be held with the patient and family to discuss this study and alternative treatments available for the underlying disease. The conference will be conducted by the outpatient-attending physician. All potential risks associated with the use of fludarabine, low dose TBI, immunosuppressive drugs, HCT, GVHD, infections, rejection, disease progression/recurrence and donor lymphocyte infusion (DLI) should be discussed as objectively as possible. It should be explained that patients offered this protocol most likely have advanced malignancy with life expectancy of months to no more than 1-2 years with conventional treatments, would be unlikely to benefit from, or tolerate an autologous transplant, and are at high risk of early transplant mortality from high dose allogeneic transplant. Informed consent from the patient will be obtained using a form approved by the Institutional Review Board (IRB) of the Fred Hutchinson Cancer Center.

9. Protocol Registration

FHCC patients: Eligible patients will be identified by the Clinical Coordinators Office. Patients will be registered with the Registration Office (206-667-4728) between 8:30 am and 4:00 PM, Monday through Friday. After hours, the Registration office can be reached by paging (206) 995-7437.

10. Plan of Treatment



A. **Randomization:** Patients will be registered at the FHCC. Please see Statistical Considerations for criteria used to randomly assign patients.

B. **HCT:** Transplant will be PBSCs collected as per NMDP or NMDP Cooperative Registry standards. One or two leukapheresis will be obtained on two consecutive days (if needed), and complete product will be infused on day "0". Because of logistical issues, PBSC infusions are usually performed in the hospital, but otherwise patients will only be admitted as medically necessary for control of transplant complications. Standard cryopreservation of a portion of PBSC will take place for potential DLI. A portion of the PBSC product will be removed for DLI that is equivalent to 3-4x10^7 CD3 cells/kg recipient weight but not more than 10% of the product and cryopreserved (see 11.I.1). If the product arrives after 6pm, 10% will be removed and held overnight prior to cryopreservation.

C. **Cytoreduction:** Cytoreduction and /or radiation therapy may be given by the referring physician or the attending physician as determined on clinical grounds or to meet eligibility requirements of the protocol for patients with advanced malignancy or to reduce tumor bulk. However, no intensive chemotherapy can be given within three weeks before conditioning (see exclusion criteria page 12). The need for this therapy should be discussed with the principal investigator. The referring oncologist may be asked to administer this therapy.

D. **Definition of Preceding Chemotherapy and Biologic Modifiers:** For the purposes of this protocol, preceding chemotherapy is defined as any exposure to systemic chemotherapy. Exceptions to this definition include BCR/ABL tyrosine kinase inhibitors (Imatinib Mesylate, Dasatinib, etc.), targeted oral agents (Idelalisib, Ibrutinib, etc), cytokine therapy, hydroxyurea, low dose cytarabine, chlorambucil, or rituxan.

E. Definition of Disease, Based on Risk of Progression: Patients will be classified as being at standard-risk, high-risk or very high risk of progression. Standard-risk includes AML in first complete remission, ALL in first complete remission, MDS-refractory anemia, CML in first chronic phase, CLL, low-grade NHL, high or intermediate grade NHL in complete remission, Hodgkin lymphoma in complete remission, multiple myeloma in complete remission or with minimal residual disease. Very high-risk includes acute leukemia beyond second complete remission, CML beyond chronic phase and MDS syndrome-refractory anemia with blast excess or above. High-risk includes all other diagnoses.

F. Pre-transplant tyrosine kinase inhibitors (Imatinib mesylate, Dasatinib, etc.).

1. All patients with the diagnosis of CML or Ph+ ALL may continue treatment with imatinib mesylate, dasatinib or other BCR/ABL tyrosine kinase inhibitors until two days prior to HCT. The tyrosine kinase inhibitors should then be stopped to prevent possible inhibition of engraftment of donor stem cells.

2. **Imatinib mesylate or dasatinib and CNS prophylaxis and treatment:** For patients who require cranial-spinal irradiation, imatinib mesylate or dasatinib will need to be discontinued 48 hours prior to initiating cranial spinal irradiation. This discontinuation is necessary because the combined effects of cranial-spinal irradiation and imatinib mesylate or dasatinib on the CNS are not known.

3. All patients with the diagnosis of CLL may continue treatment with idelalisib, ibrutinib, or other targeted oral agents until two days prior to HCT.

G. Anti-thymocyte globulin (ATG)

Use of ATG or thymoglobulin is not permitted under this protocol.

H. Conditioning Regimens:

Conditioning Regimen can consist of one of the following, at the discretion of the treating physician and will be defined prior to randomization:

Regimen A: Fludarabine (120 mg/m^2) and Melphalan (100 mg/m^2) and TBI
Patients ≥70 years of age are excluded from Regimen A.

Recommended regimen

- Fludarabine 30mg/m^2/day on Day -5 to -2 (total dose of 120mg/m2)
- Melphalan 100 mg/m^2 on day -2
- TBI 2 or 3 Gy on Day -1 or Day 0. Regardless of the actual time of TBI administration on Day 0, immunosuppression should be given per schedule relative to the infusion of PBSCs.
- Sequence of fludarabine and melphalan administration will be done according to institutional standards as long as the prescribed doses are the same

Regimen B: Fludarabine and TBI

- Fludarabine 30 mg/m^2/day IV, administered over 30 minutes on Day -4, -3, and -2 (total dose of 90 mg/m2)
- Day -1 or Day 0 TBI 2 or 3 Gy (Refer to standard practice guidelines) followed by HCT. Regardless of the actual time of TBI administration on Day 0,

immunosuppression should be given per schedule relative to the infusion of PBSCs.

a. Fludarabine

- The dose of fludarabine is based on m^2 and will use actual body weight for total administered dose of 90 mg/m^2.

- For fludarabine administered at a total dose of 120 mg/m^2, patients ≤120% of ideal body weight will be dosed according to BSA based on actual body weight. Patients >120% of ideal body weight will be dosed according to BSA based on their adjusted body weight.

- For patients receiving fludarabine total dose 120 mg/m^2 AND have an abnormal serum creatinine, fludarabine daily dose will be adjusted according to creatinine clearance. For fludarabine total dose 90 mg/m^2 no adjustment is needed for creatinine clearance.

Table 2: Fludarabine daily dose based on Creatinine Clearance	
Creatinine Clearance ml/min*	**Daily Fludarabine Dose (mg/m^2)**
> 60	30
46-60	24
31-45	22.5
21-30	19.5
≤20	15

***For fludarabine total dose 120 mg/m^2 creatinine clearance based on 24-hour urine collection is recommended for patients with abnormal serum creatinine. Assessment of creatinine clearance is recommended to be performed within 2 weeks of starting conditioning.**

b. Cyclophosphamide

i. Cyclophosphamide is dosed according to the Adjusted Body Weight if the patient's Actual Body Weight is >100% of the Ideal Body Weight.
If the Ideal Body Weight (IBW) > Actual Body Weight, then Actual Body Weight will be used.

c. Melphalan

Melphalan will be administered at a dose of 100mg/m^2. For patients >120% of ideal body weight, BSA will be calculated using adjusted weight.

d. All adjusted body weights are calculated using 25% correction.

CRITERIA FOR 3 GY TBI: Patients need to fulfill one or more of the following criteria for 3 Gy TBI:

a) Patients with MDS, MPD, CML, or other hematologic malignancies not previously treated with myelosuppressive chemotherapy
b) Patients who have had a previous allogeneic transplant.
c) Patients who had a prior syngeneic transplant without subsequent myelosuppressive chemotherapy.

d) Patients who have not had myelosuppressive chemotherapy within 3-6 months of HCT may be at higher risk of rejection depending on treatment history and underlying diagnosis. Confirm TBI dose (2 vs 3 Gy) with PI.
e) Patients with AML, ALL, or MDS with any measurable residual disease (such as by multi-parameter flow cytometry or other markers of disease including abnormalities on cytogenetics) prior to HCT
f) Patients with multiple myeloma or plasma cell leukemia

Immunosuppression Regimens:

Table 3: HLA-Matched Unrelated Donor (includes single HLA-A, -B, or –C *allele* mismatch) - Immunosuppression Schedule (Arm 1)

Day Number	-3	-2	-1	0	+1-29	+30	+40	+96	+150	+180
Stem Cell infusion				**Infusion**						
CSP (5.0 mg/kg q12hrs)	**START**	→	→	→	→	→	→	**TAPER[A]**	**STOP**	
Sirolimus (2 mg QD)*	**START**	→	→	→	→	→	→	→	**TAPER[B]**	**STOP**
MMF (15 mg/kg)				**START[C] q8hr**	→	**Q12hr**	**STOP**			

[A] CSP should only be tapered on day 96 in patients *without* preceding acute GVHD requiring therapy.
[B] The continuation of sirolimus for patients with severe acute GVHD is at the discretion of the attending physician.
[C] The first dose of MMF is to be given 4-6 hours after the stem cell infusion.
*Reduce sirolimus dose to 0.5mg QD if used concurrently with voriconazole or posaconazole

Table 4: HLA-Matched Unrelated Donor (includes single HLA-A, -B, or –C *allele* mismatch) - Immunosuppression Schedule (Arm 2)

Day Number	-3	-2	-1	0	+1-30	+40	+96	+150	+180
Stem Cell infusion				**Infusion**					
Cyclophosphamide (50mg/kg)					**Day +3[A] Day +4**				
CSP					**Day +5[B]**	→	**TAPER[C]**	**STOP**	
Sirolimus (2 mg QD)*					**Day +5[D]**	→	→	**TAPER[E]**	**STOP**

[A] The first dose of cyclophosphamide can begin approximately 62 to 72 hours after the start of the cell infusion
[B] The first dose of CSP on Day +5 should be given as an IV loading dose approximately 12-24 hours after the last dose of cyclophosphamide (See "Immunosuppression" for details).
[C] CSP should only be tapered on day 150 in patients *without* preceding acute GVHD requiring therapy.
[D] It is recommended that the first dose of sirolimus on Day +5 should be given approximately 4 hours after the CSP
[E] The continuation of sirolimus for patients with severe acute GVHD is at the discretion of the attending physician.
*Reduce sirolimus dose to 0.5mg QD if used concurrently with voriconazole or posaconazole

Table 5: HLA-Mismatched Unrelated Donor - Immunosuppression Schedule (Arm 1)

Day Number	-3	-2	-1	0	+1-29	+30	+40	+100	+150	+180	+365
Stem Cell infusion				**Infusion**							
CSP (5.0 mg/kg q12hrs)	**START**	→	→	→	→	→	→	→	**TAPER[A]**	**STOP**	
Sirolimus (2 mg QD)*	**START**	→	→	→	→	→	→	→	→	**TAPER[C]**	**STOP**
MMF (15 mg/kg)				**START[B] q8hr**	→	**Q12hr**		**TAPER**	**STOP**		

[A] CSP should only be tapered on day 150 in patients *without* preceding acute GVHD requiring therapy.
[B] The first dose of MMF is to be given 4-6 hours after the stem cell infusion.
[C] The continuation of sirolimus for patients with severe acute GVHD is at the discretion of the attending physician

Table 6: HLA-Mismatched Unrelated Donor - Immunosuppression Schedule (Arm 2)

Day Number	-3	-2	-1	0	+1-30	+40	+100	+150	+180	+365
Stem Cell infusion				**Infusion**						
Cyclophosphamide (50mg/kg)					**Day +3[A] Day +4**					
CSP					**Day +5[B]**	→	→	**TAPER[C]**	**STOP**	
Sirolimus (2 mg QD)*					**Day +5[D]**	→	→	→	**TAPER[E]**	**STOP**

[A] The first dose of cyclophosphamide can begin approximately 62 to72 hours after the start of the cell infusion
[B] The first dose of CSP on Day +5 should be given as an IV loading dose approximately 12-24 hours after the last dose of cyclophosphamide (See "Immunosuppression" for details).
[C] CSP should only be tapered on day 150 in patients *without* preceding acute GVHD requiring therapy.
[D] It is recommended that the first dose of sirolimus on Day +5 should be given approximately 4 hours after the CSP
[E] The continuation of sirolimus for patients with severe acute GVHD is at the discretion of the attending physician
*Reduce sirolimus dose to 0.5mg QD if used concurrently with voriconazole or posaconazole

3. Immunosuppression

- Day –3 (**randomized to Arm 1**). ***HLA-matched:*** Commence cyclosporine at 5.0 mg/kg PO Q12 hours, continue to day +96 and then taper until day +150. ***HLA-mismatched:*** Commence cyclosporine at 5.0 mg/kg PO Q12 hours, continue to day +150 and then taper until day +180.
- Day –-3 (**randomized to Arm 1)**. ***HLA-matched:*** Sirolimus daily dose of 2.0 mg PO QD (0.5mg PO QD if on voriconazole or posaconazole), continue to day +150 and then taper until day +180; ***HLA-mismatched:*** Sirolimus daily dose of 2.0 mg PO QD, continue to day +180 and then taper until day +365
- Day 0: **Randomized to Arm 1:** After HCT on day 0, MMF will be given based on adjusted body weight, at 15 mg/kg PO at 4-6 hours after HCT infusion is complete, then to be given at 15 mg/kg PO Q8 hours and then reduce to Q12 hours on day +30. Continue MMF Q12 hours until day +40 (***HLA-matched***) or taper at day +100 through day +150 (***HLA-mismatched***). MMF will then be discontinued without taper unless GVHD or disease relapse/progression occurs.
- Day +5 (**randomized to Arm 2**). ***HLA-matched:*** Commence cyclosporine as defined below ("Patients who will be treated with CSP beginning on day 5 after transplantation"), continue to day +96 and then taper until day +150. ***HLA-mismatched:*** Commence cyclosporine as defined below ("Patients who will be treated with CSP beginning on day 5 after transplantation"), continue to day +150 and then taper until day +180.
- Day +5 (**randomized to Arm 2**). ***HLA-matched:*** Sirolimus daily dose of 2.0 mg PO QD (0.5mg PO QD if on voriconazole or posaconazole) is recommended to start approximately 4 hours after the dose of cyclosporine, continue to day +150 and then taper until day +180; ***HLA-mismatched:*** Sirolimus daily dose of 2.0 mg PO QD (0.5mg PO QD if on voriconazole or posaconazole) is recommended to start approximately 4 hours after the dose of cyclosporine, continue to day +180 and then taper until day +365.

a. Cyclosporine

1. <u>Starting dose:</u>

   i. **<u>Adult dose</u>**: CSP is given at 5.0 mg/kg PO q12 hours according to above outlined schedule (see below for patients beginning CSP on day 5 after transplantation). Initial CSP dose is calculated using actual body weight except for those patients who are greater than 100% ideal body weight in which case calculation of dose using adjusted body weight is recommended. If there is nausea and vomiting at any time during CSP treatment the drug should be given intravenously at the appropriate dose that was used to obtain a therapeutic level. See guidelines for PO to IV conversion below.

   Patients who will be treated with CSP beginning on day 5 after transplantation: The first dose of CSP should be given approximately 12-24 hours after completion of the day +4 (second) CY infusion. A loading dose IV will be used to ensure that therapeutic blood levels of CSP are attained on day 5. Based on a terminal half-life of 19 hr, the loading dose will be 3.3 mg/kg CSP IV followed by 2.5 mg/kg CSP IV 12 hr later and every 12 hr thereafter. Cyclosporine may be switched to oral administration given at 5.0 mg/kg PO q12 hours beginning 12 hours after the loading IV dose.

2. Cyclosporine discontinuation:
   i. In the absence of acute or chronic GVHD, CSP is tapered at day 96 (HLA-matched) and 150 (HLA-mismatched) & to be completed on Day +150 or +180, respectively.
   ii. Suggested tapering schedules are as follows: HLA-matched: Decrease dose by 10-15% weekly until Day +150; HLA-mismatched: Decrease by 25% weekly until Day +180
   iii. The referring physician, who will receive instructions and guidelines for detecting and managing GVHD, may manage this. Modifications of the taper schedule may be indicated if significant disease progression occurs posttransplant. The type of modification will depend on where a patient is relative to the standard tapering schedule. Options regarding early discontinuation of CSP (and MMF) therapy are summarized below (section O).

3. Guidelines for CSP Dose Adjustment and Monitoring.
   i. Blood pressure, renal function (serum creatinine, BUN), electrolytes and magnesium need to be followed at least three times per week during the first month, twice weekly until day +100, then once per week until CSP is stopped, unless clinical circumstances suggest the need for more frequent evaluations.
   ii. CSP, whole blood "trough" levels (i.e., just prior to the next dose) will be evaluated on day 0 and twice weekly post-transplant until the initiation of the taper and adjusted if necessary
   iii. After taper, dose levels will be measured weekly if stable.
   iv. Do not exceed cyclosporine levels > 350 ng/mL when sirolimus is concurrently used to reduce risk of sirolimus toxicity.
   v. CSP Dose Adjustment: Initial high Cyclosporine (CSP) doses are required based on the pre-clinical nonmyeloablative canine studies, which used an equivalent dose to establish an allograft. After day +28, CSP levels typical for unrelated HCT will be targeted. Dose reduction should only be made if CSP toxicity is present, and/or levels exceed values provided in Table 7. There are two methods for calculating CSP levels. Table 7 provides desired levels for specific methods. To avoid inadequate immune suppression, dose reductions should be conservative. Therapeutic levels of CSP should be maintained.
   vi. After day +28, target serum CSP levels should be between 120 and 300
   vii. Dose reductions should only be made if CSP toxicity is present or levels exceed upper limits of target by 20%, depending on method (see Table 7), in the absence of toxicity. Dose reductions for high levels without toxicity should be conservative e.g. 25%, to avoid inadequate immunosuppression.
   viii. If there is nausea and vomiting at any time during CSP treatment the drug should be given intravenously at the dose that was used to obtain a therapeutic level. **Oral to IV conversion:** Oral CSP dose ÷ 2.5 = IV dose.
   ix. Oral Sandimmune may be substituted for oral Neoral.
   x. Patients requiring hemodialysis should have CSP levels maintained in the high therapeutic range (Table 7).

xi. Due to rare, potential complication of angioedema, caution should be exercised with use of ACE inhibitors in combination with sirolimus. Use of alternative agents to treat hypertension and/or proteinuria is recommended when possible.

Table 7

	CSP given with Sirolimus	
	CSP Level to Target Using LC-MS/MS Method	**CSP Level to Target Using Immunoassay Method**
Day "0"- Day +28 Whole blood "trough" (11-12 hrs from prior dose)	350 ng/ml	400 ng/ml (upper end therapeutic range for this method)
After Day +28	120 - 300 ng/ml	150 - 350 ng/ml
Levels exceeding upper limits of target by >20% • **with or without CSP toxicity** • **decrease in GFR ≥50%** • **increase in creatinine 2x baseline due to CSP**	25% dose reduction	25% dose reduction
Patients on Hemodialysis	320 ng/ml	400 ng/ml

Table 8

Decrease CSP levels	**Increase CSP levels**	**Enhance Potential for Nephrotoxicity**
Phenytoin Phenobarbital Carbamazepine Primidone Rifampicin Nafcillin Octreotide Sulfonamides Trimethoprim Metoclopramide	Erythromycin Alcohol Ketoconazole Acetazolamide Fluconazole* Colchicine Itraconazole* Fluoroquinolones Voriconazole Caspofungin Clarithromycin Diltiazem Doxycycline Verapamil Nifedipine Nicardipine Azithromycin Imipenem Posaconazole	Aminoglycosides Loop diuretics (furosemide) Amphotericin formulations

***Discontinuation of fluconazole or itraconazole may lower CSP levels, and if used for antifungal prophylaxis, then changes in these drugs should be avoided during the first 2 months posttransplant.*

b. MMF

1. Initiating MMF therapy: Oral administration of MMF will be given based on adjusted body weight at 15 mg/kg Q8 hours (45 mg/kg/day) from **the evening of day 0 (i.e. first dose to follow 4-6 hours after HCT)**. Doses will be rounded to the nearest 250 mg (capsules are 250 mg). If there is nausea and vomiting at any time preventing the oral administration of MMF, MMF should be administered intravenously based on adjusted body weight at 15 mg/kg Q8 hours.

2. MMF discontinuation:
   i. **HLA-matched randomized to MMF:** MMF will be given daily at 15 mg/kg Q8 hours until **day 30** post transplant, at and then in the absence of GVHD, the dose will be changed to 15 mg/kg Q12 hours until **day 40**. MMF will then be discontinued without taper unless GVHD or disease relapse/progression occurs
   ii. **HLA-mismatched randomized to MMF:** MMF will be given daily at 15 mg/kg Q8 hours until **day 30** post-transplant, at and then in the absence of GVHD, the dose will be changed to 15 mg/kg Q12 hours until **day 100**, then taper to **day 150** unless GVHD or disease relapse/progression occurs. A suggested taper is dose decrease by 10-15% weekly until day 150.

a. Maintaining MMF: Markedly low (<40%) donor T cell chimerism after HCT may indicate impending graft rejection. MMF should be continued at full dose or, if MMF taper has been initiated, reinstitution of full dose MMF should occur. Consideration of graft salvage with use of DLI should be considered. In the setting of acute GVHD, continuation of MMF is recommended (see **14.G** GVHD treatment guidelines).
b. Guidelines for MMF dose adjustment due to drug toxicity:
   i. If in the clinical judgment of the investigator the observed toxicity is related to MMF administration, a dose adjustment may occur. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF).
   ii. Gastrointestinal Toxicity. Severe gastrointestinal toxicities such as gastrointestinal hemorrhage have been very rare after nonmyeloablative HCT. In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea that is considered to be due to MMF after day 28, a 20% dose reduction will be made or the drug may be given IV. If severe refractory diarrhea or overt gastrointestinal bleeding occurs, MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.
   iii. Neutropenia. Based on previous experience in patients after nonmyeloablative HCT, dose adjustments are likely to occur because of hematopoietic adverse effects, in particular neutropenia. A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for grade IV neutropenia that persists after day 21post-transplant. Dose reductions should be conservative (20%). After day 21, the use of G-CSF will be permitted for neutropenia. For severe toxicity

related to MMF (grade IV neutropenia > 5 days refractory to G-CSF), MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.

c. Sirolimus

Sirolimus initiation/discontinuation:

a. Start daily dose of 2.0 mg PO daily (0.5mg PO daily if concurrently taking voriconazole or posaconazole) on Day -3 (**Arm 1**)

b. Start daily dose of 2.0 mg PO daily (0.5mg PO daily if concurrently taking voriconazole or posaconazole) on Day 5 (**Arm 2**), approximately 4 hours after CSP

c. ***HLA-matched:*** continue to day +150 and then taper until day +180

d. ***HLA-mismatched:*** continue to day +180 and then taper until day +365

   i. Suggested tapering schedules are as follows: HLA-matched: Decrease dose by 25% weekly until day +180; HLA-mismatched: Decrease dose by 5-10% weekly until day +365

2. Sirolimus dosing:

   i. Sirolimus is recommended to be given at least 4 hours after an oral dose of CSP as concurrent administration leads to elevation of sirolimus levels. In a study in renal transplant recipients, there was no significant pharmacokinetic interaction between sirolimus and CSP (45-47). However, the timing of CSP dosing affects sirolimus pharmacokinetics. Sirolimus whole-blood peak/trough levels and area under the concentration-time curve (AUC) have been significantly higher following concomitant administration of these agents compared to their administration four hours apart. Whole-blood trough levels increased by about 30% with concomitant dosing; the time to peak levels was also shorter in this group (1.8 versus 2.5 hours) (47). The most likely explanation for higher sirolimus levels during concomitant administration is an increase in sirolimus bioavailability. Clinically significant immunosuppressive synergy is observed during combined therapy with sirolimus and cyclosporine (19).

   ii. **Patients with BSA > 1.5** m^2**:** Sirolimus will be started on day –3 or +5 according to randomization at 2.0 mg every day orally through day 150 (HLA-matched) or day 180 (HLA-mismatched). **HLA-matched:** In the absence of GVHD, sirolimus should be tapered at day 150 by 25% per week for 4 weeks and discontinued on day + 180. **HLA-mismatched:** In the absence of GVHD, sirolimus should be tapered at day 180. A suggested taper schedule is dose decrease by 5-10% weekly until discontinuation on day +365. In the presence of GVHD or if the patient is receiving glucocorticoid therapy, continuation of sirolimus will be at the discretion of the attending physician or GVHD attending/team (see **14.G** GVHD treatment guidelines).

   iii. **Patients with BSA ≤ 1.5** m^2**:** For patients with BSA of ≤ 1.5 m^2, the dose will be based on BSA as follows: 1 mg/m^2/day to be rounded at the nearest 0.1mg (0.5mg PO daily if concurrently taking voriconazole or posaconazole).

iv. To minimize variability of exposure to sirolimus, the drug should be taken consistently with or without food. Grapefruit juice reduces CYP3A4-mediated metabolism of sirolimus and should not be administered with sirolimus or used for dilution.

3. Dosing will be adjusted to maintain a target blood level of 3-12 ng/mL until day 150 (HLA-matched) or day 180 (HLA-mismatched). Dose adjustments are based on clinical toxicity, blood levels, and GVHD. For levels <3 ng/mL, the dose is increased by increments of 25% until the target range is achieved. Conversely, for levels >12 ng/mL, the dose is decreased by 25% until target range is achieved. All dose adjustments will be rounded to the nearest whole number. Levels will be drawn twice weekly starting on day 0, Mondays through Fridays only. Levels should also be drawn after changing the dose of sirolimus or adding any of the medications known to interfere with the sirolimus metabolism (Appendix M).
4. The dosage will be replaced if the patient vomits within 15 minutes of taking a dose. Premedication with clinically indicated antiemetics is acceptable if vomiting occurs.
5. If there is evidence of disease progression, immunosuppression will be tapered per discretion of the treating physician.
6. Patients who are experiencing either suspected or documented fungal infection, alternative therapy should be administered whenever possible. If voriconazole, or posaconazole, are deemed necessary, sirolimus dosing reductions must be followed according to the Standard Practice Antifungal Therapy Guidelines due to contraindications.
7. Severe neutropenia or thrombocytopenia. The combination of sirolimus and cyclosporine interaction is that there is an increased risk of sirolimus toxicity such as anemia, diarrhea, hypokalemia, and thrombocytopenia. A thorough evaluation for cause of marrow suppression should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for grade IV neutropenia and thrombocytopenia that persists after day 21 post-transplant, and in the case of neutropenia is refractory to G-CSF therapy. Dose reductions of sirolimus of approximately 50% should occur. For severe persistent toxicity despite sirolimus dose reduction, sirolimus should be held until blood counts recover to ANC > 1500/µl and platelets >100,000/µl. At that point, sirolimus may be reintroduced at a 1 mg PO QD and dose increased to 2 mg/qd as long as severe hematopoietic toxicity does not occur
8. Due to rare, potential complication of angioedema, caution should be exercised for the treatment of hypertension, avoiding the use of ACE inhibitors

d. Post-transplant cyclophosphamide

On Day +3 and +4, CY will be given as a single dose of 50 mg/kg IV

Use Adjusted Body Weight for patients >100% of ideal body weight. The first post-transplant CY infusion can begin approximately 62 to 72 hours after the start of the stem cell infusion as a 1-2 hour infusion with MESNA prophylaxis and IV hydration. The Day +4 PTCy should be given approximately 24 hours after the Day +3 PTCy. Refer to standard

practice guidelines. Urine output and signs of hematuria will be monitored closely. *To maximize the effectiveness of post-transplant CY, it is critical that immunosuppressive agents are to be avoided FROM THE MORNING OF STEM CELL INFUSION until 24 hours AFTER the completion of the Day +3 post-transplant CY unless there is medical necessity.* ***This includes corticosteroids as anti-emetics****.*

For patients receiving PTCy, the start day of the PTCy and CNI may vary by one day depending on when the stem cells are infused.

I. Collection and infusions of Donor PBSC

1. G-CSF Administration to Donors: Timing of PBSC collection is prearranged through the NMDP or NMDP Cooperative Registry. G-CSF will be administered by subcutaneous injection to the unrelated donor starting 5 days prior to the day of HCT (see Table 9) as per NMDP protocol. Collection of donors after 4 days of G-CSF will be allowed. Donors will receive approximately 10 µg/kg of G-CSF each day of mobilization. Apheresis will be obtained on a one-day (Day –1 or Day 0) or a two-day (Day -1 and Day 0) collection schedule and the product will be infused on day 0. If ≤6 x 10^6 CD34 cells/kg recipient weight is collected, DLI should NOT be removed from the product.

   a. Immunophenotyping of the PBSC product for the Seattle patients will be performed by the Cellular Therapy Laboratory and will include CD34, CD3/4 and CD3/8 cells.

   b. Collection of DLI. Donor lymphocytes will be collected from unrelated donor PBSC products prior to transplant for potential future use of DLI on other protocol or treatment plans. A portion of the PBSC product from unrelated donors equivalent to 3 to 4x10^7 CD3 cells/kg recipient weight but nor more than 10% of the product may be frozen according to standard cryopreservation for DLI. If ≤6 x 10^6 CD34 cells/kg recipient weight is collected, DLI should NOT be removed from the product.

Table 9. Recommended Treatment Schema for Donor

Day	-5	-4	-3	-2	-1	0
G-CSF (~10 µg/kg)	X	X	X	X	X	[X]
PBSC collection					X	X

2. HCT Collection: HCT scheduling and collection is arranged through unrelated donor registries. The schedule of G-CSF administration and collection of PBSC is determined as per NMDP or NMDP Cooperative Registry protocol. The physician responsible for HSC collection will obtain informed consent from the donor.

3. HCT infusion: All patients will receive unmodified HCT (PBSC) infusion on day 0 of the treatment regimen (Refer to institutional practice guidelines for methods of infusion).

J. ABO incompatibility

All patients with ABO incompatibility should be evaluated and treated as according to the standard practice of the individual institution. Recommendations are provided in Appendix C. It should be noted that two cases of recipient hemolysis have been documented in patients with minor ABO mismatch with their donor. The suspected cause is donor anti-host hemagglutinin production from "passenger lymphocytes" in the donor PBSC that may expand posttransplant (48). Therefore, these patients should be monitored and treated aggressively when there is any evidence of hemolysis.

K. Post-transplant growth factors.

Patients should in general not receive post-transplant growth factors during the first 3 weeks after HCT. Growth factors should not be given unless neutropenia develops or persists past day 21 post-transplant (ANC <500/μL).

L. Post-transplant Maintenance Therapy with Tyrosine kinase inhibitors (Imatinib, Dasatinib, etc.) for Ph (+) CML or ALL patients.

Tyrosine kinase inhibitors may be reinitiated after HCT when ANC is >500/μl or on day +14 if there is no neutropenia. Tyrosine kinase inhibitor trials may also be considered.

1. **Imatinib mesylate (Gleevac)**: the suggested starting dose is:
   Patients ≥ 18 years: Imatinib at 600 mg orally each day.

2. **Dasatinib (Sprycel)**: The suggested starting dose is:
   Patients ≥18 years: Dasatinib at 70 mg orally BID (twice per day).

3. **Nilotinib (Tasigna)**: the suggested starting dose is:
   Patients ≥ 18 years: Nilotinib at 400 mg orally BID (twice per day).

   NOTE: Per FDA guidelines, patients treated with Dasatinib and Nilotinib should have hypokalemia and hypomagnesemia corrected prior to initiation in all patients.

   NOTE: Per FDA guidelines, patients treated with Nilotinib should have periodic EKG monitoring, (though not required).

   NOTE: All TKI dose reductions are allowed due to clinician judgment.

4. **Dose Reductions of Tyrosine kinase inhibitors for Grade 4 neutropenia (ANC < 500/μl) and/or thrombocytopenia (platelets < 10,000/μl) (for patients in whom platelet support is unavailable/ineffective):**

   After HCT, G-CSF will not be permitted for the first 21 days. G-CSF administration is acceptable after that time, but clinical and pathological evaluation is recommended. To assess cellularity and percentage of blasts, a bone marrow aspirate should be performed in those patients who develop Grade 4 neutropenia (ANC < 500/μl) and/or thrombocytopenia (platelets < 10,000/μl) that has lasted for ≥ 2 weeks.

a. **If the bone marrow cellularity is < 10%, and blasts < 5%,** consideration should be made to reducing the dose or holding the tyrosine kinase inhibitor therapy.
If Grade 4 neutropenia and/or thrombocytopenia persists for an additional two weeks, repeat the bone marrow aspirate to assess cellularity and percentage of blasts.
b. **If bone marrow cellularity is >10% and/or blasts >5%**, the tyrosine kinase inhibitor therapy can be increased or other therapy considered (see section 11.O.6.e).

M. Patients are eligible for trials using post-transplant therapy (such as Rituximab, FLT3 inhibitors, etc) to reduce the risk of relapse.

N. Infection Prophylaxis. Recommended prophylaxis for PCP, VZV, and HSV are listed in **Appendix E**. As antifungal prophylaxis strategies are evolving, patients may receive antifungal prophylaxis as per the standard practice of the treatment institution. Standard CMV monitoring and prophylaxis should commence at the time of initial transplant. Patients who do not become mixed or full donor chimeras can discontinue this infection prophylaxis.

O. Modifications of Immunosuppression for Low Donor T cell Chimerism, and Persistent or Progressive Disease

This section provides guidelines for management of patients with low donor chimerism and persistent or progressive disease. Those patients with significant amount of stable disease or progression of disease will undergo more rapid reduction of immunosuppression. DLI will not be given on this protocol, and patients with low chimerism or disease progression would be eligible for ongoing DLI protocols or treatment plans. Note that persistence of disease in itself does not mandate accelerated taper of immunosuppression.

1. **Definition of mixed donor/host chimerism, engraftment, graft failure and rejection**. For the purposes of this protocol, *mixed chimerism* will be defined as the detection of donor T cells (CD3+) and granulocytes (CD 33+), as a proportion of the total T cell and granulocyte population, respectively, of greater than 5% and less than 95% in the peripheral blood. *Full donor chimerism* is defined as > 95% donor CD3+ T cells. Mixed or full donor chimerism will be evidence of *donor engraftment. Increasing donor chimerism* is defined as an absolute increase of 20% of CD3+ T cells over the previous chimerism evaluation. . *Decreasing donor chimerism* is defined as an absolute decrease of 20% of CD3+ T cell chimerism over the previous month. Low donor chimerism is defined as < 40% CD3+ T cells after HCT. Low donor chimerism should always be confirmed with repeat peripheral blood T cell and granulocyte chimerism analysis. A DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or FISH studies or VNTR) of the patient and donor will be used to quantitate chimerism of sorted peripheral blood T-cells (CD3+) and granulocytes (CD 33+). The same assay should be used in a given patient for repeated studies of chimerism. This DNA-based analysis will also be performed on the whole nucleated cell fraction from marrow aspirates. Therapeutic decisions (i.e. DLI) will be made based on the results of sorted T-cell studies of *peripheral blood.* For the purposes of this protocol, *rejection* is defined as the inability to detect or loss of detection of greater than 5% donor T cells (CD3+) as a proportion of the total T cell population, respectively, after nonmyeloablative HCT. Also for the purposes of this protocol, *graft failure* is defined as grade IV thrombocytopenia and neutropenia after day 21 that lasts > 2 weeks and is refractory to growth factor support.

2. **Evaluation of chimerism** Patients will have peripheral blood and whole bone marrow evaluations for chimerism at various time points through one year post transplant. If the patient has not obtained > 95% donor chimerism in CD+3 by one year continue to evaluate through 5 years post-transplant as clinically necessary. Peripheral blood will be sorted to evaluate T-cell (CD+3), granulocyte (CD+33), **and/or** NK cell (CD+56) compartments (see Patient Post-Transplant Evaluation section for instructions and exceptions).

3. **Continuation of immunosuppression**. In the setting of low donor chimerism, immunosuppression may be continued or reinitiated at full dose so that DLI can be administered on a separate protocol. If there is disease progression in the setting of low donor chimerism, the algorithm for disease progression (below) should be followed. Patients who reject their graft may be eligible for a second allogeneic transplant on other protocols.

4. **Discontinuation of immunosuppression.** Immunosuppression should be discontinued as per protocol unless the patient develops GVHD, has falling donor chimerism or has progressive or substantial persistent disease (see below). Progressive disease may include new detection or increase in measureable/minimal residual disease, such as by flow cytometry assessment of bone marrow or other molecular methods indicative of disease progression in the opinion of the treating physician. In the setting of GVHD, CSP, MMF and sirolimus may be continued. GVHD at any time should be treated as per standard practice.

5. **Disease progression or persistence and mixed chimerism.** Evidence of substantial persistent disease at day 80 or beyond may be indication for therapeutic intervention while disease progression, at any time point will always be an indication for therapeutic intervention. Intervention for persistent disease at day 80 or beyond should be discussed with the Principal Investigator of the protocol and the guideline in Appendix F for progressive disease should be followed. If the attending physician believes that the patient requires very aggressive therapy for rapidly progressive disease, the case will be presented to the institutions' patient review committee. Otherwise, priority should be given to rapid reduction of immunosuppression, option (a) below. Therapeutic options include:

a. Discontinuation of immunosuppression. This should be considered the first therapeutic maneuver and should be tapered at the discretion of the treating physician. Bone marrow aspirate and blood chimerism studies will be performed when off immunosuppression for 2 weeks. If there is no response to stopping immunosuppression, < 20% increase in donor chimerism and there is no GVHD, patients will be considered as treatment failures. In this situation patients may receive further therapy as per institutional protocols for disease relapse or progression after allogeneic HCT. If no GVHD occurs, patients with progressive disease may be offered enrollment in institutional protocols for DLI treatment. If there is ≥ 20% absolute increase in donor chimerism, patients should be observed for additional 2 weeks and chimerism studies then repeated. If there is progressive disease that requires therapy before 4 weeks or progressive disease occurs despite onset of GVHD then patients can be treated off protocol with DLI or be considered for (b) or (c) below

b. Intercurrent treatment with chemotherapy or radiation. Conventional chemotherapy or radiation therapy should be considered in the setting of life threatening disease progression. Patients in this situation would be

considered treatment failures. After therapy is completed chimerism should be evaluated and the administration of DLI off protocol considered.
c. High dose allogeneic HCT This option should be discussed with the institutions' patient review committee and the principal investigator. Patients who undergo high dose allogeneic HCT will be removed from the protocol at that time.

11. Assessment of Disease Responses

The initial anti-tumor effect of allogeneic unrelated HCT will be evaluated with the intermittent analysis of tumor markers: Responses will be classified as complete, partial response or no response. Response criteria for MM, NHL, CLL, CML, ALL, AML and MDS to be used in this study are described in Appendix F. Standard response criteria specific to other diseases will be used in assessing disease response for other patients on study

12. Patient Evaluation

A. Patient Pre-Transplant Evaluation for All Diseases

1. History: A complete history with full details of the patient's prior treatment and response.
2. Careful physical exam with documentation of Karnofsky score, HCT CI score (**Appendix L**) and findings related to underlying malignancy.
3. CBC, creatinine, BUN, uric acid, chem 1 (Na+, K+, Cl_, Bun, creatinine, glucose), chem 2 (liver function tests), and 3(Mg+2 and Ca+2), ABO/Rh typing, hepatitis screen, CMV and toxoplasmosis serology, anti-HIV serology, and serum LDH.
4. Pulmonary function tests with corrected DLCO. When PFTs cannot be obtained, the 6MWT (also known as exercise oximetry) will be used.
5. CXR (PA and LAT).
6. ECHO or MUGA for patients > 50 years of age, or history of cardiac disease or anthracycline exposure.
7. **Evaluation and prophylaxis of CNS disease**.
   Please refer to **Standard Practice Guidelines** for recommendations for intrathecal diagnostic evaluation and prophylaxis for specific malignant diseases. In those patients that undergo intrathecal diagnostic evaluation cerebral spinal fluid should be sent for cell count and differential, cytospin, cytology, total protein, and glucose.

Immunophenotyping of the PBSC graft.
Immunophenotyping of the PBSC product for the Seattle patients will be performed by the Cellular Therapy Laboratory and will include CD34, CD3/4 and CD3/8 cells. The residual specimen will be sent to the Heimfeld lab to do phenotypic characterization of cellular subsets.

Additionally, see the following tables (Tables 10, 11, 12, 13, 14) for disease specific pre-transplant evaluations.

Table 10: Disease-Specific Pre-Transplant Evaluations for Ph (-) ALL, Ph (+) ALL, CML

Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected **within 21** days of treatment unless there is a delay. If there is a delay, and the original aspirate has no measurable residual disease, the window of time can be extended within 30 days of treatment. If there is a delay, and the original aspirate is positive for measurable residual disease, then the bone marrow must be repeated to accommodate the 21 day window. See Tables 15 and 16 for post-transplant evaluations and additional lab instructions

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	**If previously abnormal*
PCR for bcr/abl, p.210 breakpoint - **see comment*	Clinical	**CML only - reflexive testing for FHCC patients only*
PCR for bcr/abl, p.190 and p.210 breakpoints - **see comment*	Clinical	**Ph (+) ALL only - reflexive testing for FHCC patients only*
Bone marrow biopsy		
Pathology- **see comment*	Clinical	**CML only*
Peripheral Blood		
Storage for chimerism analysis	Clinical	
PCR for bcr/abl, p.210 breakpoint- **see comment*	Clinical	**CML only*

Table 11: Disease-Specific Pre-Transplant Evaluations for AML and MDS/MPD/CMML

Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected **within 21** days of treatment unless there is a delay. If there is a delay, and the original aspirate has no measurable residual disease, the window of time can be extended within 30 days of treatment. If there is a delay, and the original aspirate is positive for measurable residual disease, then the bone marrow must be repeated to accommodate the 21 day window. For MDS, in the case of a delay, the 21 day window may be extended to 30 days if there is no measurable disease on the original aspirate OR if the diagnosis is an MDS without excess blasts. See Tables 15 and 16 for post-transplant evaluations and additional lab instructions

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	**If previously abnormal*
Bone marrow biopsy		
Pathology- **see comment*	Clinical	**MDS/MPD/CMML only*
Peripheral Blood		
Storage for chimerism analysis	Clinical	

Table 12: Disease-Specific Pre-Transplant Evaluations for CLL, HL, NHL

Note: All bone marrow aspirates and biopsies are **bilateral** and must be collected within **30 days** of treatment unless there is a delay. If there is a delay, and the original aspirate has no measurable residual disease, the window of time can be extended within 45 days of treatment. If there is a delay, and the original aspirate is positive for measurable residual disease, then the bone marrow must be repeated to accommodate the 30 day window. See Tables 15 and 16 for post-transplant evaluations and additional lab instructions

Specimen / Test / Imaging	**Clinical / Research**	**Comment**
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry- **see comment*	Clinical	**No HL*
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	**If previously abnormal*
PCR for t(11:14) - **see comment*	Clinical	**Mantle Cell NHL only*
PCR for t(14:18) - **see comment*	Clinical	**Follicular NHL only*
Bone marrow biopsy		
Pathology- **see comment*	Clinical	**HL – only if history of BM involvement*
Peripheral Blood		
Storage for chimerism analysis	Clinical	
Quantitative Ig levels	Clinical	
β-2 microglobulin	Clinical	
LDH	Clinical	
Imaging		
CT of chest, abdomen, pelvis (neck if indicated)	Clinical	

Table 13: Disease-Specific Pre-Transplant Evaluations for MM and Waldenstrom's Macroglobulinemia

Note: All bone marrow aspirates and biopsies are **bilateral** and must be collected within **30 days** of treatment. See Tables 15 and 16 for post-transplant evaluations and additional lab instructions.

Specimen / Test / Imaging	**Clinical / Research**	**Comment**
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	**If previously abnormal*
Bone marrow biopsy		
Pathology	Clinical	
Peripheral Blood		
Storage for chimerism analysis	Clinical	
SPEP/IFIX	Clinical	
Quantitative Ig levels	Clinical	
β-2 microglobulin	Clinical	
Cryoglobulins, c-reactive protein, serum viscosity - **see comment*	Clinical	**Serum viscosity only for patients with >3gm/dL IgM monoclonal protein or >4gm/dL IgA or IgG protein*
Urine		
UPEP/IFIX	Clinical	
Protein / creatinine clearance	Clinical	
Imaging		
MRI – **see comment*	Clinical	**MM only*
Skeletal survey – **see comment*	Clinical	**MM only*
CT of chest, abdomen, pelvis (neck if indicated) – **see comment*	Clinical	**Waldenstrom's Macroglobulinemia only*

Table 14: Disease-Specific Pre-Transplant Evaluations for BPDCN

Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected **within 21** days of treatment unless there is a delay. If there is a delay, and the original aspirate has no measurable residual disease, the window of time can be extended within 30 days of treatment. If there is a delay, and the original aspirate is positive for measurable residual disease, then the bone marrow must be repeated to accommodate the 21 day window. See Tables 15 and 16 for post-transplant evaluations and additional lab instructions

Specimen / Test / Imaging		**Clinical / Research**	**Comment**
Bone marrow aspirate			
	Pathology	Clinical	
	Flow Cytometry	Clinical	
	Cytogenetics	Clinical	
	FISH for clonal abnormalities	Clinical	**If previously abnormal*
Bone marrow biopsy			
	Pathology	Clinical	
Peripheral Blood			
	Storage for chimerism analysis	Clinical	
Clinical skin examination by Dermatology		Clinical	
CT chest, abdomen, pelvis (neck if indicated)		Clinical	

B. Patient Post-transplant Evaluation

1. See Table 15 for disease specific post-transplant evaluation on Day +28, 56, 84, etc. This is a recommended evaluation schedule.

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Table 15: Post-Transplant Evaluation

This is a recommended evaluation schedule. See Tables 10 - 14 for pre-transplant evaluations. Additional lab instructions in Table 16.

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
Ph (-) ALL	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
Ph (+) ALL	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical		x	x	x	x	x	x	x
	FISH for bcr/abl and other clonal abnormalities	Clinical		x	x	x	x	x	x	x
	PCR for bcr/abl, p.190 and p.210 breakpoints	Clinical		x	x	x	x	x	x	x
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	PCR for bcr-abl, p.190 and p.210 breakpoints	Clinical	* If bone marrow not done and reflexive testing for FHCC patients only	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	GVHD evaluation	Clinical	See text for details			x				
AML	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
MDS/ MPD/CMML	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical	*For pts. with evidence or history of myelofibrosis	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	GVHD evaluation	Clinical	See text for details			x				
BPDCN Follow post-transplant evaluation for AML, in addition as listed here:	**Clinical skin examination**	Clinical		x	x	x	x	x	x	x
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*If abnormal pre-transplant		*See comment	*See comment	*See comment	*See comment	*See comment	*See comment

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
CML	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical		x	x	x	x	x	x	x
	FISH for bcr-abl and other clonal abnormalities	Clinical		x	x	x	x	x	x	x
	PCR for bcr-abl, and p.210 breakpoint	Clinical	*Reflexive testing for FHCC patients only	x	x	x	x	x	x	x
	BM biopsy									
	Pathology	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	PCR for bcr-abl, and p.210 breakpoint	Clinical	*If bone marrow not done and reflexive testing for FHCC patients only	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
CLL	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical				x	x	x	x	x
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	Flow cytometry	Clinical	* If peripheral blood involvement pre-transplant AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
HL - No history of BM involvement	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical				x		x		
	Cytogenetics	Clinical	*If abnormal pre-transplant			*See comment		*See comment		
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant			*See comment		*See comment		
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
HL - History of BM involvement	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical				x	x	x	x	x
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
NHL - No history of BM involvement **see separate section for additional PCR on Mantle Cell and Follicular NHLs in suspected CR*	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical				x		x		
	Flow cytometry	Clinical				x		x		
	Cytogenetics	Clinical	*If abnormal pre-transplant			*See comment		*See comment		
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	Flow cytometry	Clinical	* If peripheral blood involvement pre-transplant AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 Microglobulin	Clinical				x				
	LDH	Clinical				x	x	x	x	x
	Imaging									
	CT of chest, abdomen, pelvis (neck if indicated)	Clinical	*If abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
NHL – History of BM involvement **see separate section for additional PCR on Mantle Cell and Follicular NHLs in suspected CR*	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical				x	x	x	x	x
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	Flow cytometry	Clinical	* If peripheral blood involvement pre-transplant, if bone marrow not obtained	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical				x		x		
	LDH	Clinical			x	x	x	x	x	x
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
Mantle Cell NHL *in suspected CR*	**BM aspirate** **in addition to complete NHL restaging* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	PCR for t(11:14)	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood **in addition to complete NHL restaging*									
	PCR for t(11:14)	Clinical	*If abnormal pre-transplant, if bone marrow not obtained	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
Follicular Cell NHL *in suspected CR*	**BM aspirate** **in addition to complete NHL restaging* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	PCR for t(14:18)	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood **in addition to complete NHL restaging*									
	PCR for t(14:18)	Clinical	*If abnormal pre-transplant, if bone marrow not obtained	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
MM *Omit SPEP/IFIX and UPEP/IFIX for non-secretory MM*	**BM aspirate*** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for chrom. 13 (and other clonal) abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	SPEP and IFIX	Clinical				x	x	x	x	x
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical				x	x	x	x	x
	Cryoglobulins, C-reactive protein, viscosity	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment		*See comment
	Urine									
	Protein/creatinine clearance	Clinical				x	x	x	x	x
	UPEP and IFIX	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	Imaging									
	Complete skeletal survey	Clinical						x		x
	Skeletal MRI	Clinical						x		x
	GVHD evaluation	Clinical	See text for details			x				

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Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
Waldenstrom's Macro-globulinemia *Omit SPEP/IFIX and UPEP/IFIX for non-secretory Waldenstrom's Macro-globulinemia*	**BM aspirate****If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for chrom. 13 (and other clonal) abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	SPEP and IFIX	Clinical				x	x	x	x	x
	Quantitative Ig levels	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 microglobulin	Clinical					x	x	x	x
	Cryoglobulins, C-reactive protein, viscosity	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment		*See comment
	Urine									
	Protein/ creatinine clearance	Clinical				x	x	x	x	x
	UPEP and IFIX	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	Imaging									
	CT chest, abdomen, pelvis (neck if indicated)	Clinical	*Day 56 only if abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Table 16: Additional Lab Instructions

Note: All bone marrow tests are done on aspirate unless specifically identified as biopsy. All instructions apply to both pre- and post-transplant evaluations unless specifically identified otherwise.

Off-site providers may use local facilities for the tests.
Volumes represent desired amounts.

Specimen / Test	Type	Instructions	Lab Name	Contact Information
Bone marrow				
Chimerism	Clinical	1-3mL bone marrow in green-top tube	Clinical Immunogenetics Lab	188 East Blaine Street, Suite 250, Seattle, WA 98102 (206) 606-1139
Pathology (*aspirate*)	Clinical	2mL bone marrow in EDTA formalin	FHCC Pathology Lab	(206) 606-1355
Pathology (*biopsy*)	Clinical	1cm bone marrow in formalin **OR** mounted in paraffin	FHCC Pathology Lab	(206) 606-1355
Flow Cytometry	Clinical	2mL bone marrow in green-top tube	UW Hematopathology Lab	(206) 606-7060
Cytogenetics	Clinical	3mL bone marrow in green-top tube	FHCC Cytogenetics Lab	(206) 606-1390
FISH	Clinical	2mL bone marrow in green-top tube	FHCC Cytogenetics Lab	(206) 606-1390
PCR for bcr-abl and p190 and/or p210	Clinical	3mL bone marrow in lavender-top tube Label "protocol 9816"	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 606-7060
PCR t(11:14) or t(14:18)	Clinical	2mL bone marrow in lavender-top tube	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 606-7060
Peripheral blood				
Chimerism (CD3+), (CD33+) NK(CD56+)	Clinical	10mL blood in green-top tube for Flow sorting, then to CIL	UW Hematopathology Lab, routed to Clinical Immunogenetics Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 606-7060
Flow Cytometry	Clinical	10mL blood in green-top tube	UW Hematopathology Lab	(206) 606-7060
SPEP/IFIX	Clinical	3mL blood in red-top tube	UW Department of Laboratory Medicine	University of Washington (800) 713-5198
Quantitative Ig Levels	Clinical	3mL blood in red-top tube	FHCC Alliance Lab	(206) 606-2057
β-2 Microglobulin	Clinical	3mL blood in red-top tube	UW Department of Laboratory Medicine	University of Washington (800) 713-5198
LDH	Clinical	3mL blood in red-top tube	FHCC Alliance Lab	(206) 606-2057
PCR for bcr-abl and p190 and/or p210	Clinical	7mL blood in lavender-top tube Label "protocol 9816"	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 606-7060
PCR for t(11:14) or t(14:18)	Clinical	5mL blood in lavender-top tube	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 606-7060

Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants) for chimerism analysis.

Additionally, include the following for all diseases:

1. CBC per standard practice until day +100 or patient discharge.
2. Chem 1(Na+, K+, Cl_, Bun, Cr, glucose) and chem 3 (Mg+2, Ca+2) per standard practice until day +100 or patient discharge.

RECOMMENDED FOR ALL PATIENTS:

3. Cyclosporine trough levels on day "0" and then twice a week until taper begins. Weekly thereafter if levels are stable.
4. a) Sirolimus trough levels on day "0" and then twice a week for the first month and weekly thereafter to maintain therapeutic serum levels.

b) Serum triglyceride levels every two weeks post transplant until Day +56, then once per month until off sirolimus, or more often if clinically indicated.

c) Haptoglobin every other week until Day +56, then as indicated. Evaluation of schistocytes weekly with CBC through Day + 56.

5. Evaluate at Day +84

Patient Discharge to the Care of Referring Hematologist/Oncologist. After the day +84 work-up and screening for chronic GVHD are completed and analyzed, a patient with an uncomplicated unrelated HCT would be eligible for discharge. Since the patient may be discharged prior to starting CSP taper, instructions should be provided for preventing and detecting GVHD as per standard practice.

8. Evaluate at 1 year (+/- 1 month) for disease status (see Table 15) and chronic GVHD

The comprehensive cGHVD evaluation should be performed at FHCC (See Appendix E)
GVHD evaluation guidelines are as follows:

- History and physical exam
- Skin biopsy
- Pulmonary function test
- Oral exam
- CXR
- Dietician assessment
- Gynecological assessment (adult female)

Patients should be evaluated for GVHD per **Appendix E** prior to DLI.

9. Patients should be assessed for the need of IVIG monitoring and replacement therapy per Institutional Guidelines and patients with MM should be assessed for the need of bisphosphonates per Institutional Guidelines

C. Donor Evaluations

Unrelated donors will undergo evaluation for allogeneic hematopoietic cell donation at the collection center by NMDP standard. The attending physician of the collection center will review the results of the donor evaluation. Evaluations typically include:

1. Complete history and physical examination.

2. Lab tests: CBC with reticulocytes and platelet counts, SMAC 12, hepatitis screen, CMV, syphilis, HIV and HTLV I serologies and ABO Rh blood typing. If the donor has antibodies against red cell antigens of the recipient, the titers will be determined. Cytotoxic crossmatch between patient and donor (HLA Laboratory) will be performed.
3. No placement of a central line is necessary for G-CSF stimulated PBSC collection unless it is determined that the donor has poor venous access. If necessary, a temporary apheresis (e.g. Mahurkar) catheter will be placed at the time of leukapheresis.
4. A CBC will be checked prior to and after leukapheresis collection, and daily while on G-CSF. CBCs will be checked thereafter if clinically indicated.
5. The donor will be reevaluated the day after the apheresis is completed.

13. Drugs and Toxicities

Sirolimus, CSP, MMF and fludarabine are all commercially available. They should be stored and mixed according to manufacturer's recommendations.

A. For the purposes of this protocol, toxicity will be graded using the modified NCI common toxicity scale (**Appendix K**).

B. **TBI**: TBI will be given in one 2-3 Gy fraction (See Standard Practice Guidelines). Dosimetry calculations are performed by the radiation therapist. At the dosage used, side effects are not expected. Nevertheless, there may be fever, alopecia, parotitis, diarrhea, reversible skin pigmentation, mucositis and late effects including cataract formation, growth retardation, pulmonary damage, carcinogenesis, and sterilization.

C. **Cyclosporine**: See section **11.H.3.a.** for information about administration and dosage adjustments. Side effects are generally reversible, and may include renal insufficiency, hypomagnesemia, paresthesias, tremor, seizures, visual disturbances, paresis, disorientation, depression, confusion, somnolence, coma, nausea, hypertension, hemolytic-uremic syndrome, hyperglycemia, gynecomastia, and hypertrichosis

D. Sirolimus

1) Formulation and Administration

a. Sirolimus is supplied as oral solution (Rapamune Oral Solution) 1 mg/mL or as 1 mg tablets.

b. Rapamune Oral Solution pouches should be stored protected from light and refrigerated at 2°C to 8°C. If necessary, the patient may store the pouches at room temperatures up to 25°C (77°F) for a short period of time (e.g., several days, but no longer than 30 days). The tablets should be stored at 20-25°C and be protected from light.

c. Sirolimus is to be administered orally once daily at the doses described in Section 11.H.3.c. To minimize variability of exposure to sirolimus, this drug should be taken consistently with or without food. Grapefruit juice reduces CYP3A4-mediated metabolism of sirolimus and should not be administered with sirolimus or used for dilution.

d. If patients are receiving Rapamune Oral Solution, the dose should be mixed well with 60 mL of water or orange juice and taken immediately. It is recommended that the container be refilled with a minimum of 120 mL of water or orange juice, mixed well, and this rinse solution should be swallowed.

2) Adverse Reactions

The incidence of adverse reactions was determined in two randomized, double-blind multicenter controlled trials in which 499 renal transplant recipients received Rapamune oral solution 2 mg/day and 477 received 5 mg/day. Specific adverse reactions associated with the administration of Rapamune oral solution included hypocholesterolemia, hyperlipidemia, hypertension, and rash. At the higher dose of 5 mg, these adverse effects included anemia, arthralgia, diarrhea, hypokalemia, and thrombocytopenia. Additional toxicities from our study in stem cell transplantation include: hemolytic uremic syndrome, seizures, and neutropenia.

Appendix M lists medications including voriconazole and posaconazole that may affect metabolism of sirolimus. In patients receiving sirolimus, these drugs should be used with caution and sirolimus levels should be monitored closely. The Standard Practice Antifungal Therapy Guidelines may be used as a reference for dosing instructions**.**

3) Management of Toxicities

a. All toxicities will be scored as per common toxicity criteria (Appendix K) and unless specified in this protocol, treated as per our Standard Practice Guidelines.

b. Toxicities thought to be associated with sirolimus will be treated as follows:

i. Engraftment will be considered 3 consecutive days of ANC >500/µL on day 30. If ANC <500 on day 30 remains below 500, graft failure evaluation should be initiated as per our Standard Practice Guidelines.

ii. Severe neutropenia or thrombocytopenia. A thorough evaluation cause of marrow suppression should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for grade IV neutropenia and thrombocytopenia that persists after day 21 post-transplant, and in the case of neutropenia is refractory to G-CSF therapy. Dose reductions of sirolimus of approximately 50% should occur. For severe persistent toxicity despite sirolimus dose reduction, sirolimus should be held until blood counts recover to ANC > 1500/µl and platelets >100,000/µl. At that point, sirolimus may be reintroduced at a 1 mg po q.d. and dose increased to 2 mg/qd as long as severe hematopoietic toxicity does not occur

iii. Hyperlipidemia: Sirolimus is known to cause elevations in serum cholesterol and triglyceride levels. Serum triglyceride levelis recommended to be drawn every two weeks through Day + 56, then monthly while on Sirolimus, or more often if clinically indicated. Cholesterol levelis recommended to be drawn at Day + 84 departure workup. To avoid complications due to pancreatitis, patients should be treated with gemfibrozil, 600 mg BID p.o., or atorvastatin, 10 mg q.d. for triglyceride levels >800 mg/dL or per the discretion of the treating physician. Statins given concomitantly with sirolimus may cause CPK elevation, myalgia, or rhabdomyolysis.

E. MMF: See section **11.H.3.b** for information about administration and dosage adjustments. *Mycophenolate mofetil (MMF)*: is supplied in 250mg hard gelatin capsules. Capsules may be stored at room temperature.

i. Precautions: MMF has been studied extensively among patients after nonmyeloablative HCT. Previous clinical studies in patients after allografting suggest that the principal adverse reactions associated with the administration of MMF include nausea, vomiting, neutropenia, diarrhea, and on one occasion bloody diarrhea. In the setting of marrow transplantation, several etiologic factors may contribute to alterations in gastrointestinal and hematologic parameters. MMF has an increased incidence of digestive system adverse events, including GI tract ulceration, and hemorrhage (3% of patients receiving MMF). GI tract perforations have rarely been observed. Most patients in these studies were also on other drugs known to be associated with these complications. Up to 2% of patients receiving MMF for prevention of rejection developed severe neutropenia (ANC <500). The development of neutropenia may be related to MMF itself, concomitant medications, viral infections or some combination of these causes. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the principal investigator and documented in the medical records and the clinical reporting form (CRF). Dose adjustments are described in Section 11.H.3.b.5.

F. Fludarabine: The dose of fludarabine used in this protocol is nonmyeloablative, but does cause significant immunosuppression. Fludarabine can lower the white blood cell count, in particular the CD4+ T-cells. The immunosuppression observed with the use of fludarabine increases the risk of infection, which can be life threatening.

G. GVHD:

1. Diagnosis: Skin involvement will be assessed by biopsy with percentage of body surface area involved recorded. GI symptoms suspicious for GVHD will be evaluated by biopsy as indicated. Acute GVHD and chronic GVHD will be graded according to established criteria (**Appendix D and E**). Acute and chronic GVHD will be graded by the treating physician or LTFU.
2. Recommended Treatment:
   a. Patients developing acute GVHD ≥ grade II off immunosuppression or while on a CSP taper:
      i. CSP 5mg/kg PO q12hrs. If there is concern of GI absorption use IV route (1.5mg/kg q12hrs).
      Prednisone (2mg/kg/day) is to be added if there is no response by 72 hours or progression of GVHD during the 24 hours after the start of CSP 5.0 mg/kg PO q12hrs. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6-week steroid taper.

Patients may also be eligible for institutional trials of GVHD therapy.

   b. Patients who develop acute GVHD ≥ grade II prior to day +100:
      i. Patients who develop acute GVHD ≥ grade II should receive prednisone (1-2 mg/kg/day) or intravenous equivalent. Continuation of sirolimus beyond that defined by protocol in patients with active GVHD is at the discretion of the treating attending. A suggested sequence for immunosuppression discontinuation is as follows. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6 week steroid taper. **BOTH Arms**: When steroids are tapered to less than 0.5 mg/kg, then a

CSP taper should be initiated no sooner then day +96 and such that the completion of the taper is NOT prior to Day + 150.

- **MMF Patients:** After successful discontinuation of CSP and corticosteroids, the suggested sequence for tapering MMF is to taper the MMF such that the completion of the taper is NOT prior to Day + 40 (HLA-matched) or Day +100 (HLA-mismatched)
- If patient still receiving MMF, it should be discontinued without a taper prior to initiating a CSP taper. After successful discontinuation of MMF, CSP and corticosteroids, the suggested sequence for tapering sirolimus is to taper the sirolimus such that the completion of the taper is NOT prior to Day 180 (HLA-matched) or Day 365 (HLA-mismatched) post transplant.
- If nausea and/or vomiting prevent the oral administration of CSP or MMF, then CSP and MMF should be administered intravenously. The timing of these tapers depends on the day post transplant that acute GVHD develops, the severity of the GVHD and the clinical discretion of the attending physician.
- Patients may be eligible for institutional trials of GVHD therapy

c. Patients with clinical extensive chronic GVHD: CSP 5.0 mg/kg PO q12hrs and prednisone 1mg/kg QD or eligible protocols at the time. The patient should receive antibiotic prophylaxis with daily double strength Bactrim.

d. Patients off immunosuppression who develop concurrent manifestations of GVHD that satisfy criteria for acute GVHD ≥ grade II (e.g. erythematous rash, diarrhea, hyperbilirubinemia) **and** are pathognomonic of clinical extensive chronic GVHD (e.g. lichenoid oral changes, occular sicca, scleroderma, bronchiolitis obliterans, contractures), should receive prolonged immunosuppressive therapy similar to that for clinical extensive chronic GVHD.

H. Myelosuppression

Grade IV myelosuppression will be defined as a decrease in ANC to ≤500/uL and/or platelet count to ≤ 20,000/uL. If myelosuppression occurs, a bone marrow aspirate and biopsy should be considered to exclude disease progression. Samples should be sent for chimerism analysis by a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or FISH studies or VNTR) of the patient and donor. Myelosuppression may occur in this patient population for a number of reasons such as direct toxic effect of drugs (MMF, ganciclovir etc.), rejection, relapse or after DLI.

Patients with myelosuppression may be managed as follows:

1. Suspected MMF toxicity: refer to sections **11.H.3.b** Guidelines for MMF dose adjustment above for management recommendations.
2. Suspected sirolimus toxicity: refer to sections **11.H.3.c** for management recommendations.
3. Suspected ganciclovir toxicity: consider changing to foscarnet.
4. Patients who are > 21 days after HCT with an ANC of ≤500/uL may receive G-CSF 5μg/kg/day S.C.
5. Thrombocytopenic patients will receive platelet transfusion as per standard care.
6. Suspected BCR/ABL tyrosine kinase inhibitor therapy (such as imatinib mesylate or dasatinib) toxicity: refer to sections **11.L.4.a-b** above for management recommendations.

14. Records

Clinical records will be maintained as confidentially as possible. Collection of Case Report Forms (CRF) at standard intervals is the primary method of collecting data. Clinical Statistics at FHCC maintains a patient database to allow storage and retrieval of patient data collected from a wide variety of sources. The principal investigator will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. These data are then entered into a secure dedicated database operated by a data manager. Any publication or presentation will refer to patients by a unique patient number and not by name to assure patient confidentiality. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents.

15. Statistical Consideration and Termination of Study

This is a randomized phase II study whose primary objective is to compare chronic GVHD-free and relapse-free survival (CRFS) at 1 year after transplant between two immunosuppressive regimens. Since the two regimens differ in the number of drugs and the duration of administration, it is not feasible to blind the study; however, the assignment of GVHD severity will be performed by an independent evaluator.

All endpoints will be analyzed on an as-treated basis. For the primary endpoint (CRFS), an additional intent-to-treat analysis will be performed. The as-treated analysis will exclude patients who went off study after randomization and prior to transplant. Day 0 of the analyses will be considered the day of cell infusion (day 0 of HCT).

A. Primary Endpoint

The primary endpoint for this trial will be the composite end point of chronic GVHD-free/relapse-free survival (CRFS) in which events include moderate or severe chronic GVHD based on NIH consensus criteria (49), relapse, or death from any cause in the first post-HCT year.

B. Secondary Endpoints

Secondary endpoints are: acute (grades II-IV and III-IV) GVHD at day 100, late GVHD not meeting NIH consensus criteria for chronic GVHD, moderate and severe chronic GVHD (based on NIH consensus criteria), relapse, non-relapse mortality, progression or relapse-free survival, and overall survival at one year.

C. Sample size/Power

160 patients will be randomized to GVHD prophylaxis regimen Arm 1 or 2. The randomization will be stratified by donor HLA-match (HLA-matched vs. HLA-mismatched). Additional stratification of randomization by conditioning regimen type is not feasible; the impact of the different conditioning regimens on outcomes will be considered by multi-variate analysis. This sample size is sufficient to detect differences between regimens in a range considered to be clinically significant – which is a difference of 15%. For such a difference the power is 67% at the 2-sided 0.15 level of significance, assuming a chi-squared test. For purposes of sample size calculation, we have assumed an estimated 1-year CRFS of 45% based on results of protocol 2448 (sirolimus arm); however, the power calculations are not particularly sensitive to this rate. At a projected accrual rate of approximately 20-25 patients per year it will take 7-8 years to complete the study.

For patients who are randomized and go off protocol before transplant, data for the primary endpoint (chronic GVHD, relapse, and death) will be collected from the Gateway database in retrospective fashion.
Although for simplicity the sample size calculation is based on a binary endpoint, the actual analysis will be a time-to-event analysis of events occurring in the first year. This will take better account of patients lost to follow-up during the first year, and will be less subject to spurious results which might arise based on a single fixed time point. Additional analyses will include events occurring beyond one year.

16. Data and Safety Monitoring Plan

A. FHCC Protocol 9816 Data and Safety Monitoring Plan

1. Monitoring the progress of trials and the safety of participants

Protocol 9816 is a single-institutional clinical trial that is monitored by the principal investigator (PI) with oversight by a Data Safety and Monitoring Board (DSMB), the Data and Safety Monitoring Committee (DSMC) and the Institutional Review Board (IRB). The PI reviews outcome data for each individual patient at a minimum of 3 months after unrelated donor HCT and the updated data are presented at Mixed Chimerism Meetings (includes co-investigators).

Please see **Appendix H** for definitions of adverse events, serious adverse events (SAE) and serious and unexpected events as well as mechanisms for reporting these events. SAEs are reported to the trial coordinator. The SAE report is reviewed by the PI. If the SAE meets the FHCC criteria for expedited reporting then an official signed report is submitted to the FHCC Institutional Review Office (IRO). All deaths, regardless of the cause, are reported to the IRB. Protocol 9816 has a dedicated independent DSMB responsible for monitoring patient safety on this clinical trial. The DSMB will meet at six month intervals for this protocol and all outcome data is reviewed including all adverse events and SAEs reported to the coordinating center (FHCC) along with those officially reported to the FHCC IRO. The DSMB confirms that the trial has met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMB is submitted to the FHCC IRB as well as the trial coordinators/local PIs of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual and the last patient treated is past day +180. Furthermore, the FHCC also has a DSMC that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual IRB review and approval is also required.

Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic or referring clinicians at other institutions and is transmitted to the trial coordinator. At the FHCC, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes in the medical record. This documentation is extracted by the study nurse within 140 days +/- after HCT via chart review and collection of copies of source documents and entered into a hard copy or electronic Case Report Form (CRF). The PI reviews the official CRF and primary source documents. When the CRFs are verified, they are signed by the PI. Thus, multiple health care providers provide independent observations and participate in monitoring this trial. The PI may be a clinician for some patients entered on this trial. However, assessments are the sum total of the primary health care provider (fellow or physician assistant), floor or outpatient nurse and the PI or other attending clinician involved with the patient averting possible conflict of interest having the PI as the attending

clinician for protocol patients. If determination of adverse events is controversial, co-investigators will convene on an ad hoc basis as necessary to review the primary data and render a decision.

2. Plans for assuring compliance with requirements regarding the reporting of Serious Adverse Events SAEs

The adverse event reporting in this multi-institutional clinical trial will follow the FHCC Guidelines for SAE reporting. These guidelines (attached in **appendix G**.) detail the expedited reporting requirements, definitions of particular events. All SAEs that meet expedited criteria are reported to the IRO within 10 days by the investigator, trial coordinator, or research nurse upon learning of the event. A completed SAE report form, signed by the PI, must be received by the IRO within 10 calendar days. The PI reviews all SAEs and annual reports at the time of submission. For patients being cared for at the FHCC, health care providers communicate with the PI, trial coordinator or research nurses as events occur triggering subsequent reporting. For patients not being cared for at FHCC the outside facilities communicate with the PI, trial coordinator, or research nurse for these reporting purposes. All other deaths and expected serious adverse events are reported to the IRB at the time of annual renewal and at the biannual mixed chimerism meeting. The PI for a study is responsible for this reporting and the IRO assures adverse event reporting on an annual basis. The PI in the annual application for grant continuation will summarize reports of toxicities.

3. Plans for assuring that any action resulting in a temporary or permanent suspension of an NCI-funded clinical trial is reported to the NCI grant program director responsible for the grant

This clinical research trial uses commercial agents and there is no associated Investigational New Drug (IND) or Investigational Device Exemption (IDE). Any temporary or permanent suspension, as determined by the PI, IRB, or DSMC, of this clinical research trial will be reported to the NCI grant program director by the PI.

4. Plans for assuring data accuracy and protocol compliance

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Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). Under the provisions of this plan, Fred Hutch Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), Fred Hutch Scientific Review Committee (SRC) and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state, and federal guidelines.

17. Targeted/Planned Enrollment

**The "Ethnic Category Total of All Subjects" must be equal to the "Racial Categories Total of All Subjects."*

Ethnic Category	Gender		
	Females	**Males**	**Total**
Hispanic or Latino	12	12	24
Not Hispanic or Latino	68	68	136
Ethnic Category Total of All Subjects	80	80	160
American Indian / Alaska Native	1	1	2
Asian	4	4	8
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	10	10	20
White	64	64	128
Racial Categories: Total of All Subjects	80	80	160

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APPENDIX A
ELIGIBILITY GUIDELINES FOR DONOR PBSC APHERESIS FOR TRANSFUSION

Immunization	Donor Eligibility
Cholera	No wait
Diphtheria	No wait
Flu	24 hour wait
Gamma globulin (Immune serum globulin)	No wait unless for hepatitis
Hepatitis B vaccine	No wait unless given for hepatitis exposure
Measles (Rubella)	1 month wait
Mumps	2 week wait
Polio – Sabin (inj)	No wait
Plague	No wait
Rabies	1 year wait if given as treatment for bite. 2 week wait if given as prophylaxis (DMV's or zoo workers)
Smallpox	2 week wait
Tetanus toxoid	No wait
Typhoid	No wait
Typhus	No wait
Yellow Fever	2 week wait

APPENDIX B
THE KARNOFSKY PERFORMANCE STATUS SCALE

General	Index	Specific Criteria
Able to carry on normal activity; no special care needed	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity, minor signs or symptoms of disease
	80	Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	70	Care for self, unable to carry on normal activity or to do work
	60	Requires occasional assistance from others but able to care for most needs
	50	Requires considerable assistance from others and frequent medical care
Unable to care for self, requires institutional or hospital care or equivalent; disease may be rapidly progressing	40	Disabled; requires special care and assistance
	30	Severely disabled, hospitalization indicated, death not imminent
	20	Very sick, hospitalization necessary, active supportive treatment necessary
	10	Moribund
	0	Dead

APPENDIX C
ABO INCOMPATIBILITY

Red Blood Cell - Incompatibility (Major):
Occasional patients may have antibodies directed against red blood cell antigens found on the donor's cells. These are generally ABO or Rh antigens, although incompatibility with other red cell antigens identified by donor-recipient crossmatch may occur. Although the volume of red blood cells (RBC) in most PBMC products will only be 2-5% of the product volume before infusion, the small quantity may cause a hemolytic transfusion reaction. According to the FHCC policy it is generally acceptable to infuse a volume of about 10ml RBCs per product. If the recipient shows an anti-donor titer of ≥ 1:32 or the RBC volume is greater than 10ml (or > 20ml in two products combined) the PBMC components should be RBC depleted by Starch Sedimentation (flowsheet below). *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*
Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse red cell depleted PBMC products within 2 hours of depletion.
Expected Results: Red blood cell depleted PBMC products will contain < 10ml of red blood cells and ≥ 90% nucleated cell recovery.

Red Blood Cell - Incompatibility (Minor):
Occasional donors may have antibodies directed against red blood cell antigens (ABO, Rh, or other antigen system) found on the recipient's cells. The risk of hemolysis of recipient red cells immediately after transplant is not of very much clinical import. Due to the high number of lymphocytes in the PBMC inoculum, recipients may be at much greater risk for a delayed type of hemolysis that can be severe. PBMC products contain < 200ml of plasma according to FHCC policy and no deleterious effects have been observed so far. However, if donors show an anti-recipient titer ≥ 1:256, the PBMC component should be plasma depleted (see flowsheet below). *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*
Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse plasma-depleted PBMC within 2 hours of depletion.
Expected Results: The plasma depletion should not affect the nucleated cell recovery.

Red Blood Cell – Bidirectional Incompatibility:
Patients undergoing transplants for bidirectional RBC incompatibility should be managed according to both algorithms shown below. Most red cell depletion techniques also deplete plasma from the PBMC component with no additional cell loss. *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

Post transplant blood component support will be according to Standard Practice Guidelines.

MAJOR ABO INCOMPATIBLE

Recipient anti-Donor titer	≥ 1:32	<20ml RBC total	⟹	Infuse without modification
		>20ml RBC total	⟹	RBC depletion of component
	≤ 1:16		⟹	Infuse without modification

MINOR ABO INCOMPATIBLE

Donor anti-Recipient titer	≥ 1:256	Plasma depletion of component
	≤ 1:128	Infuse without modification

APPENDIX D
GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE [a]

Severity of Individual Organ Involvement		
Skin	+1	a maculopapular eruption involving less than 25% of the body surface
	+2	a maculopapular eruption involving 25-50% of the body surface
	+3	generalized erythroderma
	+4	generalized erythroderma with bullous formation and often with desquamation
Liver	+1	bilirubin (2.0-3.0 mg/100 ml)
	+2	bilirubin (3-5.9 mg/100 ml)
	+3	bilirubin (6-14.9 mg/100 ml)
	+4	bilirubin > 15 mg/100 ml
Gut	Diarrhea is graded +1 to +4 in severity. Nausea and vomiting and/or anorexia caused by GVHD is assigned as +1 in severity The severity of gut involvement is assigned to the most severe involvement noted. Patients with visible bloody diarrhea are at least stage +2 gut and grade +3 overall	
Diarrhea	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	+2	>1,000 ml of stool/day* (> 15ml of stool/kg/day)†
	+3	>1,500 ml of stool/day* (> 20ml of stool/kg/day)†
	+4	2,000 ml of stool/day* (≥ 25ml of stool/kg/day)†

**In the absence of infectious/medical cause*
†For pediatric patients

Severity of GVHD	
Grade I	+1 to +2 skin rash
	No gut or liver involvement
Grade II	+1 to +3 skin rash
	+1 gastrointestinal involvement and/or +1 liver involvement
Grade III	+2 to +4 gastrointestinal involvement and/or
	+2 to +4 liver involvement with or without a rash
Grade IV	Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death

a From "Graft-vs-host disease" Sullivan, Keith M. **Hematopoietic Cell Transplantation** ***Ed: D. Thomas, K. Blume, S. Forman, Blackwell Sciences; 1999, pages 518-519***

APPENDIX E
CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD)

Chronic GVHD in allogeneic transplant recipients resembles autoimmune disorders such as scleroderma, Sjogren syndrome, primary biliary cirrhosis, lichen planus, wasting syndrome, bronchiolitis obliterans among others manifestations (see below). Approximately 50% of patients will develop this complication within 6 months after the transplant despite continued treatment with immunosuppressive medications. Close monitoring is recommended during the first 2 years after allogeneic stem cell transplantation so that appropriate treatment can be instituted promptly in patients who develop chronic GVHD. Debilitation, joint contractures and profound immunosuppression resulting in recurrent bacterial infections are prominent characteristics of untreated chronic GVHD.

A. Grading of Chronic GVHD
Chronic GVHD severity will be classified according to the 2014 NIH consensus criteria (49).

NIH Global Severity of chronic GVHD (49)

Mild
- 1 or 2 organs involved with no more than score 1 plus
- Lung score 0

Moderate
- 3 or more organs involved with no more than score 1 OR
- At least 1 organ (not lung) with a score of 2 OR
- Lung score 1

Severe
- At least 1 organ with a score of 3 OR
- Lung score of 2 or 3

Late GVHD NOT meeting NIH 2014 criteria for chronic GVHD
- Any GVHD (gut, liver, skin) after Day 100 that does NOT have any chronic manifestations (diagnoses)
- This would be a secondary endpoint event

Overlap GVHD by NIH 2014 criteria
- Any GVHD that has BOTH gut, liver, skin that clinically resembles acute GVHD AND any chronic manifestations (diagnoses)
- This would be a primary endpoint event

Chronic GVHD by NIH 2014 criteria
- Any GVHD that has NO gut, liver, skin that clinically resembles acute GVHD, but has any chronic manifestations (diagnoses)
- This would be a primary endpoint event

	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, capabl of self-care, >50% of waking hours ou 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† ☐ **SCORE % BSA**				
GVHD features to be scored by BSA: **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration

Other skin GVHD features (NOT scored by BSA)
Check all that apply:
☐ Hyperpigmentation
☐ Hypopigmentation
☐ Poikiloderma
☐ Severe or generalized pruritus
☐ Hair involvement
☐ Nail involvement
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ 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

☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS**				
Symptom score:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **%** FEV1 ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____				

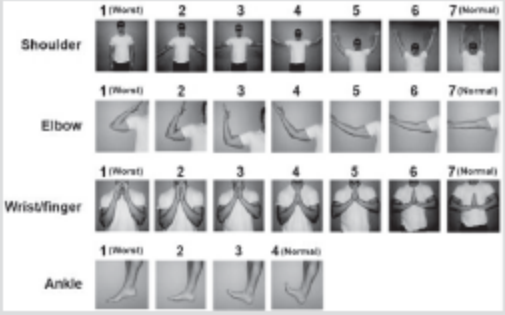
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought 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.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have symptoms with discomfort on exam	☐ Severe signs‡ with or without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

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

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Photographic Range of Motion (P-ROM)



Above table from Jagasia et al. Biol Mlood Marrow Transplant 21 (2015) 389-401 (49)

B. Physical manifestations of Chronic GVHD

Manifestations that are distinctive for chronic GVHD can begin before day 100 after the transplant, and manifestations that are typical of acute GVHD can persist long after day 100. For this reason, the differential diagnosis between acute and chronic GVHD cannot be made solely according to the time interval from transplant. The diagnosis of chronic GVHD requires at least one manifestation that is distinctive for chronic GVHD (*identified by italic print below*) as opposed to acute GVHD. In all cases, infection and others causes must be ruled out in the differential diagnosis of chronic GVHD.

Karnofsky or Lansky Clinical Performance scores <60%, ≥15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ system are listed below (*italic print identifies manifestation more distinct of chronic GVHD):*

Skin	Erythema, dryness, pruritis, macular-papular or urticarial rash, *pigmentary changes (i.e., hyperpigmentation, vitiligo), mottling, papulosquamous or lichenoid plaques, hyperkeratosis, exfoliation (ichthyosis), nodules, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)*. The extent of skin involvement and the skin thickness score for patients with scleroderma needs to be recorded at the time of diagnosis, when changes in treatment are made and when assessing treatment response. Medical photos are also useful for assessing the extent of skin involvement and response to treatment.
Nails	**B. Ridging, onychodystrophy, onycholysis**
Hair	*Premature graying (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair*
Mouth	Dryness, burning, gingivitis, mucositis, striae, *dryness, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tightness around the mouth, sensitivity to acidic, strong flavors, heat or cold, tooth decay*
Eyes	*Dryness, burning, blurring, gritty eyes, photophobia, pain*
Vagina/vulva	*Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not induced by ovarian failure or other causes*
Liver	Jaundice and elevated liver function tests not due to other causes (see laboratory tests)
Lung	*Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis*
GI	Anorexia, nausea, vomiting, diarrhea, *malabsorption, dysphagia, odynophagia*
Myofascial	*Stiffness and tightness with restriction of movement, occasionally with swelling, pain, cramping, erythema and induration, most commonly affecting the forearms, wrists and hands, ankles, legs and feet, inability to extend the wrists without flexing the fingers or the elbows, contractures*
Muscle	**Proximal muscle weakness, cramping**
Skeletal	*Arthralgia of large proximal girdle joints and sometimes smaller joints*
Serosal	*Unexplained effusions involving the pleural, pericardial, or peritoneal cavities not due to venocclusive disease of the liver, cardiac insufficiency, malignancy, infection, GM-CSF toxicity or other causes*

C. Laboratory Testing and Diagnostic Indicators of Chronic GVHD

Eye	*Schirmer's test with a mean value ≤ 5 mm at 5 minutes, or values of 6-10 mm in patients who have sicca symptoms, or keratitis detected by slit lamp examination*
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase ≥2 x upper limit, of normal, AST or ALT >3 x upper limit of normal or total serum bilirubin ≥1.6)

Lung	*New obstructive lung defect defined as an FEV_1 <80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV_1/FVC by >12% within a period of less than 1 year, thought not to be caused by an infectious process, asthma or recurrent aspiration from the sinuses or from gastroesophageal reflux. In the absence of GVHD in any other organ, the diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage, evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scan of the lungs, or confirmation by thoracoscopic biopsy.*
Esophagus	*Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry*
Intestine	Endoscopic findings of mucosal edema and erythema or focal erosions with histological changes of apoptotic epithelial cells and crypt cell drop out. Patients with unresolved acute GVHD may have more severe intestinal mucosal lesions including ulcers and mucosal sloughing.
Muscle	*Elevated CPK or aldolase, EMG findings consistent with myositis with biopsy revealing no other etiological process*
Blood	Thrombocytopenia (usually 20,000-100,000/µl), eosinophilia (> 0.4 x 10^3/uL), hypogammaglobulinemia. Hypergammaglobulinemia and autoantibodies occur in some cases.

D. Guidelines for Treatment of Chronic GVHD after allogeneic HCT

We strongly recommend that you consult the LTFU office before beginning treatment for chronic GVHD and before making changes in immunosuppressive treatment. Clinical trials should always be considered because current standard therapies are associated with high morbidity and decreased survival for patients with high risk chronic GVHD.

Standard treatment of chronic GVHD usually begins with administration of glucocorticoids (1mg/kg/day) followed by taper to eventually reach an alternate-day regimen, with or without daily cyclosporine or tacrolimus (FK506). Other medications used for treatment of corticosteroid-resistant chronic GVHD are summarized on the next page. Telephone consultation with the LTFU medical team is available to you, seven days a week, to discuss appropriate treatment and provide other follow up recommendations. In addition to immunosuppressive treatment, antibiotic prophylaxis for encapsulated bacterial infections and PCP must be given to all patients being treated for chronic GVHD (see Standard Practice Guidelines).

The duration of systemic immunosuppressive treatment of chronic GVHD varies but requires at least one year of therapy. Approximately 80% of patients require systemic immunosuppressive for 2 years and 40% of them requires therapy for at least 4 years.

Adapted From: Long-Term Followup After Hematopoietic Stem Cell Transplant General Guidelines For Referring Physicians, Fred Hutchinson Cancer Center Standard Practice Manual, Section X, Chronic Graft Versus Host Disease (GVHD), Nov/2003 Version

APPENDIX F
EVALUATION OF DISEASE RESPONSE

Chronic myeloid Leukemia (CML)

Complete response:	Normalization of the white count with complete disappearance of the Ph chromosome in 20 out of 20 metaphases whenever possible. Molecular response is defined by negative RT-PCR for the BCR/ABL transcripts in bone marrow or blood.
Partial response:	Normalization of the white count with >0% but ≤35% Ph metaphases.
No response:	Persistence of ≥80% Ph-positive metaphases.
Progressive disease:	Acquisition of a new cytogenetic abnormality and/or development of accelerated phase or blast crisis. The criteria for accelerated phase will be defined as unexplained fever greater than 38.3° C, new clonal cytogenetic abnormalities in addition to a single Ph-positive chromosome, marrow blasts and promyelocytes in access of 20%.

Acute leukemia (AML, ALL)

Complete response:	<5% marrow blasts by pathology and no circulating leukemic blasts.
Partial response:	5-30% marrow blasts, or <5% marrow blasts with circulating blasts.
Stable disease:	>30% marrow blasts without definite deterioration of performance status or worsening of anemia, neutropenia, or thrombocytopenia.
Progressive disease:	Evidence of relapse (>5% blasts) by morphologic or flow cytometric evaluation of the bone marrow aspirate or appearance of extramedullary disease

Chronic lymphocytic leukemia (CLL)

Complete remission:	Normal imaging studies (X-ray, CT, MRI) (nodes, liver, and spleen), peripheral blood by flow cytometry has no clonal lymphocytes, bone marrow by flow cytometry has no clonal lymphocytes, bone marrow by morphology has no nodules (or if present, nodules are free from CLL cells by immunohistochemistry), and the duration is ≥2 months.
CR with minimal residual disease:	Peripheral blood or bone marrow by flow cytometry >0 - <1 CLL cells/1000 leukocytes (0.1%)
Partial remission:	Absolute lymphocyte count in peripheral blood ≥50% decrease[3] and physical exam/Imaging studies (nodes, liver, and/or spleen) ≥50% decrease[3, 4]. Duration is ≥2 months.
Progressive disease:	≥1 of: Physical exam/imaging studies (nodes, liver, and/or spleen) ≥50% increase or new, circulating lymphocytes by morphology and/or flow cytometry ≥50% increase, and lymph node biopsy with Richter's transformation
Stable disease:	Did not meet any of the above criteria for complete or partial remission or progression.
Relapsed disease:	Criteria of progression occurring 6 months after achievement of complete or partial remission.

Lymphoma [Hodgkin's Disease, Non-Hodgkin's Lymphoma (NHL)]

Complete response:	Disappearance of all clinically detectable disease.
Partial response:	≥50% reduction of the sum of the products of the perpendicular diameters of marker lesions, no progression of any existing lesions, and no new lesions.
Stable disease:	Stabilization of all existing lesions with no new lesions (i.e. a <25% increase or <50% decrease in disease parameters defined above throughout the treatment period).
Progressive disease:	>25% increase in the sum of the products of the perpendicular diameters of marker lesions, or the appearance of new lesions.

Multiple Myeloma (MM)

Complete response:	Disappearance of plasmacytomas; decrease in marrow plasmacytosis to less than 10%; ≥75% reduction of the monoclonal serum protein. Reduction of the 24 hour urine M-component to 10% or less of the initial prestudy value and to less than 0.2 gm/day; no increase in the size or number of lytic skeletal lesions; and normal serum calcium.
Partial response:	≥50%, <75% reduction of the monoclonal serum protein and reduction of the 24 hour urine M-component to less than 0.2 gm/day; no increase in serum calcium, or in the size or number of plasmacytomas or lytic skeletal lesions.
Stable disease:	<50% reduction or <100% increase of the serum myeloma protein.
Progressive Disease:	≥100% increase of the serum myeloma protein from its lowest level, or reappearance of myeloma peaks that had disappeared with treatment; or definite increase in the size or number of plasmacytomas or lytic bone lesions.

Myelodysplasia (MDS)

Progressive Disease:	Any evidence by morphologic or flow cytometric evaluation of the bone marrow aspirate of new blasts (>5%).

[1] Without granulocyte colony stimulating factor support.
[2] Without red blood cell transfusions or erythropoietin support.
[3] Compared to before starting therapy.
[4] Defined by the sum of the products of up to 6 lymph nodes with no increase in the size of any single lymph node (ie, an increase of <25 percent in a lymph node <2cm is not considered significant) and no new enlarged lymph nodes.

1. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, Rai KR. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 87: 4990-4997, 1996.

2. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, Kipps TJ, International Workshop on Chronic Lymphocytic Leukemia. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines [Erratum appears in Blood. 2008 Dec 15;112(13):5259]. Blood 111: 5446-5456, 2008.

3. Chronic lymphocytic leukemia: recommendations for diagnosis, staging, and response criteria. International Workshop on Chronic Lymphocytic Leukemia. Ann Intern Med 110: 236-238, 1989.

APPENDIX G
STUDY COORDINATOR'S MANUAL

I. Introduction

The mixed chimerism protocols have been opened to multiple sites to increase the referral base and accrual. Because of this expansion of collaborators, the data collection procedures are being revised. The procedure manual was created to assure consistency of data reporting across the centers and to assure compliance with regulations. General expectations of collaborators are that they will comply with appropriate regulatory requirements, specified protocol requirements, and provide outcome data.

The manual translates working procedures for study coordination. Its goal is to describe the procedures with sufficient clarity to ensure that all study centers will use the same procedures and follow-up schedules for participant data management and reporting. Changes to the manual and relevant forms will be made as soon as practical and will become effective on receipt of the revised procedures at the study centers, unless otherwise noticed.

II. Institutional Review Board Review of Protocols and Modifications

All research protocols proposed for use that involves human subjects must be reviewed and approved by the Institutional Review Board (IRB) prior to implementation. New protocols will undergo review at the FHCC IRB and then will be distributed to sites that wish to participate for their IRB's review. For Centers that have a Federal Wide Assurance (FWA), formal collaboration includes submission of a form 310 and a copy of the IRB approved protocol and consent forms to the FHCC. For sites without a FWA, an FWA form needs to be filed. Once the paperwork is submitted to the Office for Human Research Protection, the approval process can take up to a couple of months, and must be completed before collaboration on a protocol can begin.

In addition, all amendments and/or revisions to on-going, approved activities must be submitted for review and approved prior to implementation at an institution. No revisions may be implemented at outside institutions without the prior approval of the FHCC Principal Investigator. The FHCC and the local site's IRB must review all protocol activities at least once annually. This must be done within 365 days of the last review regardless of the policies of the institution. A copy of annual renewal approvals must be received for collaboration to continue for the next year.

III. Reporting Adverse Events

The following guidelines are the minimum serious adverse event (SAE) reporting guidelines for Category 1 and 2 studies conducted at the Fred Hutchinson Cancer Center.

Expedited Reporting Requirements

All adverse events (whether occurring on-site or off-site), which in the opinion of the principal investigator are (1) unexpected, and (2) related or possibly related to the research and (3) serious or suggests that the research places research participants or others at a greater risk of physical or psychological harm than was previously known or recognized must be submitted to the IRB within ten (10) calendar days of becoming aware of the event.

Definitions

An Adverse Event - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related.

Related or Possibly Related Adverse Event: An adverse event is "related or possibly related to the research procedure" if in the opinion of the principal investigator, it was more likely than not caused by the research procedures. Adverse events that are **solely** caused by an underlying disease, disorder or condition of the subject or by other circumstances unrelated to either the research or any underlying disease, disorder or condition of the subject are not "related or possibly related." If there is any question whether or not an adverse event is related or possibly related, the adverse event should be reported.

Serious Adverse Event: An adverse event that results in any of the following outcomes: Death, a life-threatening adverse event (real risk of dying), inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity/or change in psychosocial status, a congenital anomaly or, requires intervention to prevent permanent impairment or damage.

Unexpected Adverse Event: An adverse event is "unexpected" when its nature (specificity), severity, or frequency are not consistent with (a) the known or foreseeable risk of adverse events associated with the research procedures described in the protocol-related documents, such as the IRB-approved research protocol, informed consent document and other relevant sources of information such as product labeling and package inserts; and are also not consistent with (b) the characteristics of the subject population being studied including the expected natural progression of any underlying disease, disorder or condition or any predisposing risk factor profile for the adverse event.

To ensure no confusion or misunderstanding exist of the differences between the terms "serious" and "severe," which are not synonymous the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) or a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is *not* the same as "serious," which is based on patient/event *outcome or action* criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory obligations.

For example, hospitalization, in general, will not be considered a serious adverse event as approximately half of evaluable MRD patients AND the majority of evaluable URD patients receiving non-myeloablative transplants were hospitalized. Hospitalization will be considered a serious adverse event if it fulfills the criteria for a serious and unexpected adverse event as described above.

Serious events, including deaths, due to GVHD and/or infections will not be reported on an expedited basis. These are well documented, expected, post transplant complications and will be reported biannually to the DSMB.

It is the responsibility of the FHCC Principal Investigator to notify the sponsor, NIH, FDA or other agencies of serious adverse events as required in the protocol.

Reporting of Adverse Events on Case Report Forms (CRF)
All grade 3 or 4 adverse events (or highly unusual grade 2 adverse events), which occur between the start of any protocol intervention and day 100 during the study will be recorded on the CRF. These adverse events which are observed by the Investigator or reported by the patient, whether or not attributed to the study, will be reported on the Case Report Form using the selected (for this protocol) NCI Common Toxicity Criteria (NCI-CTC) version 4.03 (**Appendix L**). Attributes will include a description, date of onset, maximum severity, and assessment of relationship to the study agent or other suspect agent(s). These grade 3 or 4 adverse events will be reported to the DSMB as part of the biannual review of the protocol. The DSMB report is submitted with the annual IRB renewal.

Reporting of Unanticipated Problems that Involve Risk to Research Participants or Others:
Any incident, experience, or outcome that meets both of the following criteria:

- Unexpected (in terms of nature [specificity], severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Indicates that the research places research participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

These must be reported to the FHCC Investigator within 10 days of learning of the event as described above for reporting of SAE.

IV. Case Report Forms

Case report forms must be completed for all patients registered onto the protocol and submitted to the FHCC data coordinating center. When possible, primary source documents regarding patient outcomes are collected with patients' names removed and replaced by Unique Patient Numbers (UPNs). The CRFs are generated at defined time points (day 28, 56, 84, 100, 6 months, 1 year, 18 months and annually). The PI reviews the official CRF and primary source documents. When the CRFs are verified, the data is entered into a central database managed by the trial coordinator.

V. Protocol Monitoring

As the coordinating center, FHCC will monitor accrual at the outside institutions. The guidelines below are intended to guide the reviewers in their assessment of items that significantly alter the clinical effectiveness of the treatment or the evaluation of its toxicity.

A. Registration/Randomization
   1. Patient was registered prior to treatment and approval by FHCC PI occurs prior to randomization.
   2. Information given at registration represents actual data in medical records (stage, diagnosis, cell type, etc.)

B. Informed Consent/IRB Approval Dates
   1. The consent was signed prior to registration

2. The consent is in language was approved by the institution's IRB. IRB approval and reapproval are documented including appropriate use of full-board review and proper review of appropriate amendments or revisions
3. Consent was dated and has written witness signature. IRB approval was obtained prior to the patient signing the consent form and start of treatment.

C. Patient Eligibility
1. Eligibility criteria and exclusion criteria were met
2. Treatment/Intervention Administration
3. Doses were modified according to protocol
4. Accurate documentation of drug administration

D. Study Tests/Evaluation
1. Protocol specified laboratory tests or diagnostic studies are available
2. Appropriate record of protocol intervention is documented.

E. Study Events/Adverse Drug Experience
1. Serious Adverse Evens reported according to protocol specifications

F. Follow-Up
1. Disease status assessed according to the required protocol guidelines documenting response to treatment.
2. Accurate determination of cancer progression

APPENDIX H

Fred Hutchinson Cancer Center

SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08

FHCC IR File Number: _____ FHCC Protocol Number: _____

FHCC Unique Patient # _____ ☐ FHCC ☐ Other

Gender: ☐ Male ☐ Female Age: _____

FHCC Principal Investigator: _____

Phone Number: _____ Mailstop: _____

Date of Report: _____

☐ Initial Report _____ ☐ Follow-Up Report # _____ ☐ Other

Date Study Staff became aware of event: _____

Date Serious Adverse Event Started: _____

Date Ended: _____ Or ☐ Ongoing (if ongoing – must submit follow up report)

Adverse Event: _____

_____

Describe the Serious Adverse Event including a summary of all relevant clinical information. (Or attach a MedWatch Form or other SAE reporting form if one has been completed.) Use Page 2, if necessary: _____

_____

Outcomes Attributed to adverse event: (Check all that apply)

☐ Death ___/___/_____
☐ Life-Threatening
☐ Hospitalization (initial or prolonged)
☐ Disability
☐ Congenital Anomaly
☐ Required intervention to prevent permanent impairment/damage

Specify Agent(s) and/or Procedure(s) involved in this protocol:

#1 _____

Pharmaceutical product/medical treatment/procedure
☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

#2 _____

Pharmaceutical product/medical treatment/procedure
☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

☐ Follow-up Report Required ☐ Final Report (PI must sign final report)

Report Completed by: Date:

_____ _____

The PI has determined that the consent form must be revised: ☐ Yes ☐ No

Does this study involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer)? ☐ yes ☐ no If yes and the activity involves the FHCC outpatient clinic, a copy of this Protocol Modification Form and any supporting documents to be reviewed and approved, will be forwarded to the FHCC's Institutional Biosafety Committee (IBC) by the Protocol Office (Mailstop: LM-230).

_____ Date: _____

Signature of Principal Investigator

Fred Hutchinson Cancer Center
Clinical Research Division
Institutional Review Office
SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08
page 2

FHCC IR File Number: _____ FHCC Protocol Number: _____

FHCC Unique Patient # _____ Date of Report: _____

Describe the Serious Adverse Event including a summary of all relevant clinical information.

APPENDIX I
NOTICE OF DEATH

Patient ID: _____ Date of Death: _____

Place of Event: _____

Apparent cause of death (Please be specific. Attach hospital summary or death summary when possible):

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Form completed by: _____Date: _____

APPENDIX J

Protocol **9816** Patient Demographics and Eligibility Form
Please Fax this completed form to (206) 667-5378 for patient registration.
Questions regarding eligibility should go to Masumi Ueda Oshima, MD 206-667-4546.

UPN#:_____

Patient Name: _____ _____ _____
(Last) *(First)* *(MI)*

Date of Birth: _____ / _____ / _____ **Age**:_____
(Mo) *(Day)* *(Year)*

Gender *(choose one):*
☐ Male ☐ Female ☐ Unknown

Patient Diagnosis: _____

Planned Day 0: _____/_____/_____
(Mo) *(Day)* *(Year)*

Ethnicity *(choose one): Instruct the patient to select one of the following.*

- ☐ **Hispanic** *(A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. Term "Spanish Origin" can also be used in addition to "Hispanic" or "Latino".)*
- ☐ **Not Hispanic or Latino**
- ☐ **Declined to Report**

Race *(check all that apply): Instruct the patient to select one or more of the following.*

- ☐ **American Indian/Alaska Native** *(A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment).*
- ☐ **Asian** *(A person having origins in any of the original peoples of the Far East, Southeast, Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand and Vietnam).*
- ☐ **Black/African American (***A person having origins in any of the black racial groups of Africa).*
- ☐ **Native Hawaiian/Pacific Islander** *(A person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands).*
- ☐ **White** *(A person having origins in any of the original peoples of Europe, the Middle East or North Africa).*
- ☐ **Research subject does not know race**
- ☐ **Declined to report**

CRITERIA FOR 3 GY TBI: Patients need to fulfill one or more of the following criteria for 3 Gy TBI:

☐ Patients with MDS, MPD, CML, or other hematologic malignancies not previously treated with myelosuppressive chemotherapy
☐Patients who have had a previous allogeneic transplant.
☐Patients who had a prior syngeneic transplant without subsequent myelosuppressive chemotherapy.
☐ Patients who have not had myelosuppressive chemotherapy within 3-6 months of HCT may be at higher risk of rejection depending on treatment history and underlying diagnosis. Confirm TBI dose (2 vs 3 Gy) with PI.
☐ Patients with AML, ALL, or MDS with any measurable residual disease (such as by multi-parameter flow cytometry or other markers of disease including abnormalities on cytogenetics) prior to HCT
☐ Patients with multiple myeloma or plasma cell leukemia

TBI Dose:
☐ TBI 2 Gy
OR
☐ TBI 3 Gy:

Donor:
☐ HLA-MATCHED (includes single HLA-A, -B, or -C *allele* mismatch)
a) Matched for HLA-A, B, C, DRB1 and DQB1 by high resolution typing;
b) ***Only a single allele disparity*** will be allowed for HLA-A, B, or C as defined by high resolution typing

☐ HLA-MISMATCHED
a) Mismatch for one HLA class I antigen with or without an additional mismatch for one HLA-class I allele, but matched for HLA-DRB1 and HLA-DQ, OR
b) Mismatched for two HLA class I alleles, but matched for HLA-DRB1 and HLA-DQ
c) HLA class I HLA-A, -B, -C allele matched donors allowing for any one or two DRB1 and/or DQB1 antigen/allele mismatch

Signature of **FHCC** Research Staff**:** _____ Date: _____

Signature of **Local** Principal Investigator**:** _____ Date: _____

Transplant Center: _____

Signature of **FHCC** Principal Investigator: _____ Date: _____

FHCC Use Only

Study UPN#: _____

Internal B#:_____

Randomization Arm

HLA-MATCHED
☐ Arm 1: CSP, SIR, MMF
☐ Arm 2: CSP, SIR, PTCy

HLA-MISMATCHED
☐ Arm 1: CSP, SIR, MMF
☐ Arm 2: CSP, SIR, PTCy

_____ _____
Signature **Date**

APPENDIX J cont'd

Protocol 9816 Eligibility

I) Inclusion Criteria:

1) Yes ☐ No ☐ Unrelated donors who are prospectively:

**FHCC matching allowed will be Grades 1.0 to 2.1 (See Standard Practice Guidelines).

a) Matched for HLA-A, B, C, DRB1 and DQB1 alleles by high resolution typing **AND**

b) Only a single allele disparity will be allowed for HLA-A, B, or C as defined by high resolution typing (See Standard Practice Guidelines for other donor selection details) **OR**

Mismatched with the recipient within one of the following limitations:

a) Mismatch for one HLA class I antigen with or without an additional mismatch for one HLA-class I allele, but matched for HLA-DRB1 and HLA-DQ, OR

b) Mismatched for two HLA class I alleles, but matched for HLA-DRB1 and HLA-DQ

c) HLA class I HLA-A, -B, -C allele matched donors allowing for any one or two DRB1 and/or DQB1 antigen/allele mismatch

Yes ☐ No ☐ Have a negative anti-donor cytotoxic crossmatch.

NA ☐ Cytotoxic crossmatch **not done as patient and donor are phenotypically identical by molecular methods.**

Yes ☐ No ☐ Patient and donor pairs are not homozygous at a mismatched allele in the graft rejection vector.

NA ☐ Patient and donor are 10/10 HLA-allele matched by high resolution typing.

☐ **PI has reviewed HLA report of** _____
date

One of the following criteria questions (2-5) must be marked "Yes" for the patient to enter on 9816.

2) Yes ☐ No ☐ Ages >50 years with hematologic malignancies treatable by allogeneic HCT.

3) Yes ☐ No ☐ Ages 18 to 50 years with hematologic diseases treatable by allogeneic HCT who through pre-existing medical conditions or prior therapy are considered to be at high risk for regimen related toxicity associated with a high dose transplant

Pre-existing condition(s) precluding high dose tx: _____

4) Yes ☐ No ☐ Ages 18 to 50 years with chronic lymphocytic leukemia (CLL).

5) Yes ☐ No ☐ Ages 18 to 50 years with hematologic diseases treatable by allogeneic HCT who refuse a high dose HCT. Transplants must be approved for these inclusion criteria by the principal investigator.

II) One of the following criteria questions (6-16) must be marked "Yes" for the patient to enter on 9816. (The following diseases will be permitted although other diagnoses can be considered if approved by PCC the principal investigator.)

6) Yes ☐ No ☐ **Aggressive nonHodgkin lymphomas (NHL) and Other Histologies Such as Diffuse large B cell NHL**– not eligible for autologous HCT, not eligible for high dose myeloablative HCT, or after failed autologous HCT.

7) Yes ☐ No ☐ **Mantle Cell NHL** -may be treated in first CR (Diagnostic LP required pre-transplant)

8) Yes ☐ No ☐ **Low grade NHL**– with < 6 month duration of CR between courses of conventional therapy.

9) Yes ☐ No ☐ **CLL** – must have either 1) failed to meet NCI Working Group criteria for complete or partial response after therapy with a regimen containing FLU (or another nucleoside analog, e.g. 2-CDA, pentostatin) or experience disease relapse within 12 months after completing therapy with a regimen containing FLU (or another nucleoside analog); 2) failed FLU-CY-Rituximab (FCR) combination chemotherapy at any time point; or 3) have "17p deletion" cytogenetic abnormality. Patients should have received induction chemotherapy but could be transplanted in 1st CR; or 4) Patients with a diagnosis of CLL (or small lymphocytic lymphoma) or diagnosis of CLL that progresses to prolymphocytic leukemia (PLL), or T-cell CLL or PLL. 5) Patients failing to achieve a response to ibrutinib as first-line therapy; 6) Patients not responding to ibrutinib, idelalisib, or venetoclax as salvage therapy or intolerant of these agents as salvage therapy due to side effects. All CLL patients must have received prior myelosuppressive chemotherapy.

Describe which inclusion is specific for this patient:_____
_____.

10) Yes ☐ No ☐ **Hodgkin lymphoma** – must have received and failed frontline therapy.

11) Yes ☐ No ☐ **Multiple Myeloma** – must have received prior chemotherapy. Consolidation of chemotherapy by autografting prior to nonmyeloablative HCT is permitted.

12) Yes ☐ No ☐ **Acute Myeloid Leukemia (AML)** – must have < 5% marrow blasts at the time of transplant.

13) Yes ☐ No ☐ **Acute Lymphocytic Leukemia (ALL)** – must have <5% marrow blasts at the time of transplant.

14) Yes ☐ No ☐ **Chronic Myeloid Leukemia (CML)** – Patients in CP1 must have failed or be intolerant of TKIs. Patients beyond CP1 will be accepted if they have <5% marrow blasts at time of transplant.

15) Yes ☐ No ☐ **Myelodysplasia (MDS)/Myeloproliferative Syndrome (MPS)/CMML** –Patients must have <5% marrow blasts at time of transplant.

16) Yes ☐ No ☐ **Waldenstrom's Macroglobulinemia –** must have failed 2 courses of therapy.

17) Yes ☐ No ☐ **Mixed Phenotype Acute Leukemia (MPAL)** – must have < 5% marrow blasts at the time of transplant.

18) Yes ☐ No ☐ **Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) – must be in complete remission at the time of transplant**

III) Exclusion criteria:

Each of the following questions must be marked "No" Or "NA" for the patient to enroll on 9816.

17) Yes ☐ No ☐ NA ☐ Patients with rapidly progressive intermediate or high grade NHL.

18) Yes ☐ No ☐ NA ☐ Patients with a diagnosis of CMML who have not received induction chemotherapy.

19) Yes ☐ No ☐ NA ☐ Patients with MDS-EB or AMLreceiving conditioning Regimen B (Fludarabine and TBI) who have not received myelosuppressive chemotherapy i.e. induction chemotherapy or at least 4 cycles of a venetoclax-containing regimen

20) Yes ☐ No ☐ CNS involvement with disease refractory to intrathecal chemotherapy. For LP requirement, see Standard Practice Guidelines.

21) Yes ☐ No ☐ NA ☐ Presence of circulating blasts determined to be associated with disease (in the peripheral blood) for patients with AML, ALL or CML.

22) Yes ☐ No ☐ NA ☐ Presence of ≥ 5% circulating leukemic blasts (in the peripheral blood) detected by standard pathology for patients with MDS/MPS/CMML

23) Yes ☐ No ☐ NA ☐ Fertile men or women unwilling to use contraceptive techniques during and for 12 months following treatment.

24) Yes ☐ No ☐ NA ☐ Females who are pregnant or breast-feeding.

25) Yes ☐ No ☐ Patients with active non-hematological malignancies (except non-melanoma skin cancers) or those with non-hematological malignancies (except non-melanoma skin cancers) who have been rendered with no evidence of disease, but have a greater than 20% chance of having disease recurrence within 5 years.
This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy.

26) Yes ☐ No ☐ Fungal infections with radiological progression after receipt of amphotericin B or active triazole for greater than 1 month.

NOTE: The FHCC PI of the study must approve of enrollment of all patients with pulmonary nodules.

PI Signature: _____Date: _____

The starting dose for sirolimus for patients receiving voriconazole or posaconaozle is 0.5mg daily with further adjustment based on sirolimus levels according to Standard Practice Antifungal Therapy Guidelines.

27) Yes ☐ No ☐ Cytotoxic agents for "cytoreduction" with the exception of tyrosine kinase inhibitors (such as imatinib), cytokine therapy, hydroxyurea, low dose cytarabine, chlorambucil, or rituxan will not be allowed within three weeks of the initiation of conditioning

28) Yes ☐ No ☐ **Organ dysfunction.** Please check yes if patient meets any of the following.

Yes ☐ No ☐ **Cardiac:** ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of < 26%). Ejection fraction is required if age > 50 years or there is a history of anthracycline exposure or history of cardiac disease.

NOTE: If shortening fraction is <26%, a cardiology consult is required. The PI of the study must approve eligibility

PI Signature: _____ **Date**:

Yes ☐ No ☐ **Pulmonary:** DLCO < 40%, FEV1 <40% and/or receiving supplementary continuous oxygen. When PFTs cannot be obtained, the 6-minute walk test (6MWT, also known as exercise oximetry) will be used: Any patient with oxygen saturation on room air of <89% during a 6MWT will be excluded.

Yes ☐ No ☐ **Liver function abnormalities**: Patients with clinical or laboratory evidence of liver disease would be evaluated for the cause of liver disease, its clinical severity in terms of liver function, and the degree of portal hypertension. Patients will be excluded if they are found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bridging fibrosis, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3 mg/dL, or symptomatic biliary disease.

29) Yes ☐ No ☐ Karnofsky score < 60

30) Yes ☐ No ☐ Patient has poorly controlled hypertension and on multiple antihypertensives.

31) Yes ☐ No ☐ HIV positive patients.

32) Yes ☐ No ☐ Active bacterial or fungal infections unresponsive to medical therapy.

33) Yes ☐ No ☐ Patients on hemodialysis.

Note – the HCT-Comorbidity score is: _____

☐ **FHCC Patients:**

Signature of person completing form: _____ Date: _____

Signature of Principal Investigator (eligibility confirmed and pre-randomization):

_____ Date: _____

Patient signed IRB approved consent form. Date: _____

IRB file number: _____

Signature of Principal Investigator (post signing of consent and post-randomization):

_____ Date: _____

APPENDIX K
COMMON TOXICITY CRITERIA (CTC)
Version 4.03

Grade			
Adverse Event	**3**	**4**	**5**
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Disseminated intravascular coagulation	Laboratory findings and bleeding	Life-threatening consequences; urgent intervention indicated	Death
Febrile neutropenia	ANC <1000/mm3 with a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4 degrees F) for more than one hour	Life-threatening consequences; urgent intervention indicated	Death
Hemolysis	Transfusion or medical intervention indicated (e.g., steroids)	Life-threatening consequences; urgent intervention indicated	Death
Hemolytic uremic syndrome	Laboratory findings with clinical consequences (e.g., renal insufficiency, petechiae)	Life-threatening consequences, (e.g., CNS hemorrhage or thrombosis/embolism or)	Death
Grade			
Adverse Event	**3**	**4**	**5**
CARDIAC DISORDERS			
Atrial fibrillation	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
Atrial flutter	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
Atrioventricular block complete	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening consequences; urgent intervention indicated	Death
Constrictive pericarditis	Symptomatic heart failure or other cardiac symptoms, responsive to intervention	Refractory heart failure or other poorly controlled cardiac symptoms	Death
Heart failure	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated	Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)	Death

Left ventricular systolic dysfunction	Symptomatic due to drop in ejection fraction responsive to intervention	Refractory or poorly controlled heart failure due to drop in ejection fraction; intervention such as ventricular assist device, intravenous vasopressor support, or heart transplant indicated	Death
Myocardial infarction	Severe symptoms; cardiac enzymes abnormal; hemodynamically stable; ECG changes consistent with infarction	Life-threatening consequences; hemodynamically unstable	Death
Myocarditis	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated	Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)	Death
Pericardial effusion	Effusion with physiologic consequences	Life-threatening consequences; urgent intervention indicated	Death
Pericardial tamponade	-	Life-threatening consequences; urgent intervention indicated	Death
Ventricular arrhythmia	Medical intervention indicated	Life-threatening consequences; hemodynamic compromise; urgent intervention indicated	Death
Grade			
Adverse Event	**3**	**4**	**5**
GASTROINTESTICAL DISORDERS			
Ascites	Severe symptoms; invasive intervention indicated	Life-threatening consequences; urgent operative intervention indicated	Death
Diarrhea	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Duodenal ulcer	Severely altered GI function; TPN indicated; elective operative or endoscopic intervention indicated; limiting self care ADL; disabling	Life-threatening consequences; urgent operative intervention indicated	Death

Gastric ulcer	Severely altered GI function; TPN indicated; elective operative or endoscopic intervention indicated; limiting self care ADL; disabling	Life-threatening consequences; urgent operative intervention indicated	Death
Gastritis	Severely altered eating or gastric function; TPN or hospitalization indicated	Life-threatening consequences; urgent operative intervention indicated	Death
Lower gastrointestinal hemorrhage	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Mucositis oral	Severe pain; interfering with oral intake	Life-threatening consequences; urgent intervention indicated	Death
Oral hemorrhage	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Pancreatitis	Severe pain; vomiting; medical intervention indicated (e.g., analgesia, nutritional support)	Life-threatening consequences; urgent intervention indicated	Death
Typhlitis	Symptomatic (e.g., abdominal pain, fever, change in bowel habits with ileus); peritoneal signs	Life-threatening consequences; urgent operative intervention indicated	Death
Grade			
Adverse Event	**3**	**4**	**5**
GENERAL DISORDERS AND ADMINISTARTION SITE CONDITIONS			
Multi-organ failure	Shock with azotemia and acid-base disturbances; significant coagulation abnormalities	Life-threatening consequences (e.g., vasopressor dependent and oliguric or anuric or ischemic colitis or lactic acidosis)	Death
Grade			
Adverse Event	**3**	**4**	**5**
HEPATOBILIARY DISORDERS			
Cholecystitis	Severe symptoms; radiologic, endoscopic or elective operative intervention indicated	Life-threatening consequences; urgent operative intervention indicated	Death
Grade			
Adverse Event	**3**	**4**	**5**
IMMUNE SYSTEM DISORDERS			

Allergic reaction	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated	Death
Immune system disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
		Grade	
Adverse Event	**3**	**4**	**5**
INFECTIONS AND INFESTATIONS			
Enterocolitis infectious	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic, endoscopic, or operative intervention indicated; profuse watery diarrhea with signs of hypovolemia; bloody diarrhea; fever; severe abdominal pain; hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Infections and infestations - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
		Grade	
Adverse Event	**3**	**4**	**5**
INVESTIGATIONS			
Alanine aminotransferase increased	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Aspartate aminotransferase increased	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Blood bilirubin increased	>3.0 - 10.0 x ULN	>10.0 x ULN	-

Carbon monoxide diffusing capacity decreased	Asymptomatic decrease of >8 units drop; >5 units drop along with the presence of pulmonary symptoms (e.g. , >Grade 2 hypoxia or >Grade 2 or higher dyspnea)	-	-
Cardiac troponin I increased	Levels consistent with myocardial infarction as defined by the manufacturer	-	-
Cardiac troponin T increased	Levels consistent with myocardial infarction as defined by the manufacturer	-	-
Creatinine increased	>3.0 baseline; >3.0 - 6.0 x ULN	>6.0 x ULN	-
Weight gain	>=20% from baseline	-	-
		Grade	
Adverse Event	**3**	**4**	**5**
METABOLISM AND NUTRITIONAL DISORDERS			
Hypercalcemia	Corrected serum calcium of >12.5 - 13.5 mg/dL;>3.1 - 3.4 mmol/L; Ionized calcium >1.6 - 1.8 mmol/L; hospitalization indicated	Corrected serum calcium of >13.5 mg/dL; >3.4 mmol/L; Ionized calcium >1.8 mmol/L; life-threatening consequences	Death
Hypertriglyceridemia	>500 mg/dL - 1000 mg/dL; >5.7 mmol/L - 11.4 mmol/L	>1000 mg/dL; >11.4 mmol/L; life-threatening consequences	Death
Hyperuricemia	>ULN - 10 mg/dL (0.59 mmol/L) with physiologic consequences	>10 mg/dL; >0.59 mmol/L; life-threatening consequences	Death
Tumor lysis syndrome	Present	Life-threatening consequences; urgent intervention indicated	Death
		Grade	
Adverse Event	**3**	**4**	**5**
NEOPLASMS BENIGN, MALIGNANT, AND UNSPECIFIED (INC CYSTS AND POLYPS)			
Treatment related secondary malignancy	Non life-threatening secondary malignancy	Acute life-threatening secondary malignancy; blast crisis in leukemia	Death
		Grade	
Adverse Event	**3**	**4**	**5**
NERVOUS SYSTEM DISORDERS			
Dysarthria	Severe impairment of articulation or slurred speech	-	-
Intracranial hemorrhage	Ventriculostomy, ICP monitoring, intraventricular thrombolysis, or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Ischemia cerebrovascular	-	-	-

Leukoencephalopathy	Severe symptoms; extensive T2/FLAIR hyperintensities, involving periventricular white matter involving 2/3 or more of susceptible areas of cerebrum +/- moderate to severe increase in SAS and/or moderate to severe ventriculomegaly	Life-threatening consequences; extensive T2/FLAIR hyperintensities, involving periventricular white matter involving most of susceptible areas of cerebrum +/- moderate to severe increase in SAS and/or moderate to severe ventriculomegaly	Death
Seizure	Multiple seizures despite medical intervention	Life-threatening; prolonged repetitive seizures	Death
Syncope	Fainting; orthostatic collapse	-	-
Nervous system disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Grade			
Adverse Event	**3**	**4**	**5**
RENAL AND URINARY DISORDERS			
Chronic kidney disease	eGFR or CrCl 29 - 15 ml/min/1.73 m2	eGFR or CrCl <15 ml/min/1.73 m2; dialysis or renal transplant indicated	Death
Renal and urinary disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Grade			
Adverse Event	**3**	**4**	**5**
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
Grade			
Adverse Event	**3**	**4**	**5**
RESPIRATORY, THORACIC, AND MEDIASTINAL DISORDERS			
Adult respiratory distress syndrome	Present with radiologic findings; intubation not indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Apnea	Present; medical intervention indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death

Bronchopulmonary hemorrhage	Transfusion, radiologic, endoscopic, or operative intervention indicated (e.g., hemostasis of bleeding site)	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Hypoxia	Decreased oxygen saturation at rest (e.g., pulse oximeter <88% or PaO2 <=55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Pleural effusion	Symptomatic with respiratory distress and hypoxia; surgical intervention including chest tube or pleurodesis indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Pneumonitis	Severe symptoms; limiting self care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Pulmonary edema	Severe dyspnea or dyspnea at rest; oxygen indicated; limiting self care ADL	Life-threatening respiratory compromise; urgent intervention or intubation with ventilatory support indicated	Death
Respiratory failure	-	Life-threatening consequences; urgent intervention, intubation, or ventilatory support indicated	Death
		Grade	
Adverse Event	**3**	**4**	**5**
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Erythema multiforme	Target lesions covering >30% BSA and associated with oral or genital erosions	Target lesions covering >30% BSA; associated with fluid or electrolyte abnormalities; ICU care or burn unit indicated	Death
		Grade	
Adverse Event	**3**	**4**	**5**
VASCULAR DISORDERS			
Capillary leak syndrome	Severe symptoms; intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Hypotension	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated	Death
Thromboembolic event	Thrombosis (e.g., uncomplicated pulmonary embolism [venous], non-embolic cardiac mural [arterial] thrombus), medical intervention indicated	Life-threatening (e.g., pulmonary embolism, cerebrovascular event, arterial insufficiency); hemodynamic or neurologic instability; urgent intervention indicated	Death
Vasculitis	Severe symptoms, medical intervention indicated (e.g., steroids)	Life-threatening; evidence of peripheral or visceral ischemia; urgent intervention indicated	Death

Appendix L
THE HEMATOPOIETIC CELL TRANSPLANT-COMORBIDITY INDEX (HCT-CI) 9/7/10

Assign scores appropriately if the patient has any of these comorbidities

Patient _____ *(name), UPN*_____ *Date*_____

Comorbidities	Definitions	HCT-CI scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventricular arrhythmias requiring treatment *in the patient's past history*	1	
Cardiac	Coronary artery disease†, congestive heart failure, myocardial infarction *in patient's past history* or EF of ≤50% *at time of HCT*	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis requiring treatment *in the patient's past history*	1	
Diabetes	Requiring treatment with insulin or oral hypoglycemic, but not diet alone, *at time of HCT*	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular accident *in patient's past history*	1	
Psychiatric disturbance	Depression/anxiety requiring psychiatric consult or treatment *at time of HCT*	1	
Hepatic – mild	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN *at time of HCT*	1	
Obesity	Patients with a BMI of >35 for adults or with BMI-for-age percentile of ≥ 95th percentile for children *at time of HCT*	1	
Infection	Documented infection or fever of unknown etiology requiring anti-microbial treatment *before, during and after* the start of conditioning regimen	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica *in patient's past history*	2	
Peptic ulcer	Requiring treatment *in patient's past history*	2	
Renal	Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation *at time of HCT*	2	
Moderate pulmonary	DLco and/or FEV_1 >65%-80% or Dyspnea on slight activity *at time of HCT*	2	
Prior solid tumor	Treated at any time point *in the patient's past history*, excluding non-melanoma skin cancer	3	
Heart valve disease	*At time of HCT* excluding mitral valve prolapse	3	
Severe pulmonary	DLco and/or FEV_1 ≤65% or Dyspnea at rest or requiring oxygen *at time of HCT*	3	
Moderate/severe hepatic	Liver cirrhosis, Bilirubin >1.5 X ULN, or AST/ALT >2.5XULN *at time of HCT*	3	
Please provide (KPS):	**Karnofsky Performance Score = _____%**	***Total Score*** =_____	**Signature of Provider:** _____

†One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft. 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; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Appendix M
CLINICALLY SIGNIFICANT INDUCERS/INHIBITORS OF CYTOCHROME P450 ENZYME SYSTEM

Agents <u>likely to *increase*</u> Rapamycin (Sirolimus) levels	**Agents <u>which may *increase*</u> Rapamycin (Sirolimus) levels**	**Agents <u>likely to *decrease*</u> Rapamycin (Sirolimus) levels**	**Agents <u>which may *decrease*</u> Rapamycin (Sirolimus) levels**
Diltiazem Nicardipine Verapamil Erythromycin Ketoconazole Voriconazole Clarithromycin	Cimetidine	Carbamazepine Phenobarbital Phenytoin Rifampin	Primidone Valproic acid Rifabutin

*Fluconazole, itraconazole, CSP, methylprednisolone, and tacrolimus may increase levels