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A Phase II Study to Assess Immunosuppression with Sirolimus Combined with Cyclosporine (CSP) and Mycophenolate mofetil (MMF) for Prevention of Acute GVHD after Non-myeloablative HLA Class I or II Mismatched Donor Hematopoietic Cell Transplantation– A Multi-Center Trial

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2. Introduction

Hematopoietic cell transplantation (HCT) after nonmyeloablative conditioning is a major curative strategy for patients with hematologic malignancies, who are ineligible for high dose conditioning because of age or medical infirmities (1,2). One of the most important factors associated with the successful outcome of allogeneic HCT is the extent of HLA matching between patient and donor. Although these observations mainly stem from high dose conditioning HCT (3-8), similar observations of HLA mismatch being associated with graft rejection, graft-versus-host disease and non-relapse mortality, have been observed in smaller studies of patients transplanted after nonmyeloablative or reduced intensity conditioning HCT (9,10). FHCRC protocol 1591 was a phase I/II clinical trial designed to extend the availability of nonmyeloablative conditioning HCT with 90 mg/m^2 and 2 Gy TBI to patients with HLA class I mismatched donors (11). To prevent graft rejection and provide additional GVHD prophylaxis patients were treated with cyclosporine (CSP) and mycophenolate mofetil (MMF) for extended periods of time compared to the standard care for patients transplanted with unrelated HLA matched donors. Of the 59 patients who were enrolled in the study only 2 rejected, showing that sustained engraftment is attainable in HLA mismatched HCT after minimal conditioning. However, rates of GVHD (grade II-IV acute GVHD 69%, grade III-IV acute GVHD 26%, extensive chronic GVHD 41%) were high with a two-year non-relapse mortality at 47%.

The primary goal of the proposed protocol is to determine whether the incidence of grade II-IV acute GVHD after non-myeloablative HLA class I or II mismatched donor HCT can be reduced to less than the historical rate of 70%, by extending the immunosuppressive regimen to encompass sirolimus in addition to CSP and MMF. Sirolimus has previously been shown to be effective as prophylaxis after HLA-matched and mismatched unrelated HCT (12-17), and recently in FHCRC protocol 1938, superior GVHD control was observed with a combination of sirolimus, MMF and tacrolimus compared to MMF and tacrolimus alone.

3. Background data

3A. HLA class I or II-mismatched myeloablative transplant

HLA class I (HLA-A, B, C) mismatching stimulates CD8+ T cells, whereas HLA class II (HLA-DR, DP, DQ) mismatching stimulates CD4+ T cells. Theoretically each pathway of T cell stimulation can cause both graft-versus-host reactions and host-versus-graft reactions. Clinical studies evaluating the role of HLA disparity have shown that both HLA class I and II disparities have similar adverse impacts on outcome in unrelated HCT. However, they have shown that the magnitude of the contribution of HLA class I and II disparities to GVHD or graft rejection is not equal. Furthermore, recent reports show that the risk of graft failure, GVHD and mortality are correlated with the number of HLA inconsistencies in HLA class I, class II and the combination of class I and II.

Within the past decade, high resolution typing techniques have been developed to allow identification of polymorphic alleles among class I and class II HLA antigens. When donors that were matched for HLA-A and –B by serologic typing and for HLA-DRB1 were typed retrospectively by DNA techniques, only half were matched at the allele level for all 5 loci (HLA-A, B, C, DRB1, DQB1), and one-quarter were mismatched for multiple alleles (4). The ability to distinguish allele level mismatches has allowed investigation of the relevancy of patient-donor mismatching. Subsequent studies show that impact of patient-donor mismatching depends on the disease being treated, and within disease risk groups depends upon the degree of HLA mismatch and the locus of HLA mismatch.

Initial studies of patients with chronic myeloid leukemia (CML) found an increased risk for graft failure when donors had multiple mismatches that involved at least one class I allele 29% when the mismatch involved more than one class I allele mismatch and 12% for mismatched pairs involving both class I and class II alleles (p=0.003 and 0.01, respectively), compared to 2% or less when either no mismatch or a mismatch confined to a single HLA-A, B, C, DRB1 and DQB1 allele was present (3-6). The highest risk for severe acute GVHD was observed for multiple mismatches involving both class I and class II alleles (2.0 hazard ratio, p=0.02). Pairs with a single class I mismatch did not have a significant increase in acute GVHD compared with matched recipients, but a single class II mismatch or multiple class I mismatches both appeared to confer a higher (though not significant) hazard of severe GVHD. An important limitation of these studies was that patients were mainly Caucasian, therefore results may not be transferable to other ethnic populations. For example, studies from Japan found that mismatching of HLA-A and B, but not class II HLA, decreased survival (5).

The effects of HLA-mismatch on outcome have been shown by several sequential analyses conducted through the National Marrow Donor Program. The first analysis of 1874 patient-donor pairs examined the endpoints of engraftment, acute GVHD, chronic GVHD and mortality according to locus of donor-recipient HLA disparity (7). Disparity of class I HLA loci HLA-A, -B, -C, and HLA-DRB1 were independently associated with a statistically significant increase in the risk for mortality. In addition, mismatching at HLA-A was associated with higher risk for both acute and chronic GVHD. In this study, class I HLA allele mismatches (detectable only with high-resolution typing) did not appear to increase the risk for poor outcome. This study employed multivariate modeling whereas the subsequent NMDP/CIBMTR study used subset analysis (8). In this study of 3,857 AML, ALL, CML, and MDS patients, a single mismatch for HLA-A, B, C, or DRB1 was associated with a higher risk for TRM and acute GVHD, and mismatch for HLA-A, C, and DRB1, but not HLA-B, was associated with statistically worse survival compared to the 8 of 8 HLA allele matched pairs. In contrast to the previous NMDP report, the effect of a mismatch at the allele level was equivalent to a serologic or antigen mismatch. A donor with multiple HLA mismatches increased the risk for mortality, in a degree dependent fashion, hazard ratio 1.25 and 1.65 ($p<0.0001$) for 7 and 6 of 8 loci mismatch, respectively compared to 8 of 8 matched pairs. The Japanese Marrow Donor Program analysis also supports the idea that disparities involving HLA-class I alleles are independent risk factors for acute GVHD, TRM, and overall survival. In the Japanese study, the addition of HLA-C allele disparity with other HLA allele mismatches increased the risk of acute GVHD in a synergistic fashion (5,18). HLA class I allele mismatches also were associated with a significantly higher incidence of graft failure when compared to patients with allele matched donors.

Most patients included in the sequential retrospective studies of high-resolution HLA matching received bone marrow grafts. Compared to marrow, G-CSF-mobilized peripheral blood stem cells (PBSC) contains on average 10-fold more CD3+ cells and 4-fold higher CD34+ cells, as well as differences in the relative contribution of cell subsets (19). Accordingly the NMDP/CIBMTR recently completed an analysis of the effects of HLA-mismatch among 1933 recipients of unrelated PBSC grafts. HLA-mismatched pairs that contained at least one antigen mismatch had statistically worse survival (RR 1.23, 95% CI 1.12-1.55, p=0.0007) and disease-free survival (RR 1.54, 95% CI 1.10-1.51, p=0.0013) compared to pairs who were 8/8 matched; however, survival of 7/8 allele mismatches was not statistically different than 8/8 matched pairs. Locus-specific analysis of single mismatched pairs found that mismatch of a single HLA-C antigen was associated with a statistically significant higher risk for mortality, TRM and grade III-IV acute GVHD and lower disease-free survival. At two years, survival was 32% for HLA-C mismatches compared to 44% of 8/8 matches

(p=0.003). DFS was 26% compared to 40% (p=0.003), and TRM was 40% compared to 28% (p=0.002), respectively. Mismatching at a single HLA-B allele or antigen also was associated with increased grade III-IV acute GVHD. The risks of relapse and chronic GVHD, were not statistically different for recipients of 8/8 matches compared to any locus-specific mismatch, including HLA-C antigen mismatched pairs.

In the myeloablative setting, the number of tolerated mismatches appears to differ according to the disease being treated. In the Seattle analysis of marrow recipients, patients with early stage disease had a significant decrease in survival and increase in NRM when the graft was mismatched for a single antigen or allele (20). In contrast, the relevance of HLA mismatching among intermediate-stage or advanced-stage patients could be appreciated only when two or more disparities existed (HR 1.40, CI 1.08-1.82). The Lee study also showed a relatively greater negative effect of HLA-mismatching for patients according to disease stage: early > intermediate> advanced (8).

3B. HLA-mismatched Transplantation with minimal or reduced toxicity conditioning

Most data that is available on the role of HLA class I or II mismatches in allogeneic HCT are derived from patients transplanted after high dose conditioning. Apart from our own experience from FHCRC protocol 1591, only few studies deal with the specific question of HLA mismatch in the nonmyeloablative setting. However, data is to some extent attainable from studies of mainly HLA-matched cohorts which include smaller subsets of mismatched transplants.

In a retrospective study published by Teshima et al (9), data was reported from a cohort of 341 transplant patients, which included 57 single HLA-locus mismatches (5/6) and 34 two or three loci mismatches (3-4/6). Patients were transplanted for hematological malignancies with PBSC from related donors after reduced intensity conditioning. The most frequently used conditioning regimens were fludarabine-based (150–180 mg/m^2 with either cyclophosphamide 60 mg/kg, busulphan 8 mg/kg or melphalan 80–140 mg/m^2) with or without TBI 2–4 Gy or ATG 5–10 mg/kg. The most frequently used regimens for GVHD prophylaxis were CSP alone or CSP plus MTX. Patient and donor pairs were serologically typed for HLA-A, -B and –DR, and if a mismatch was observed, subsequent intermediate level DNA typing was performed for all three loci. In patients with a 6/6 matched donor the incidence of graft failure was 3.7% (N=9), while it was 5.7% (N=3) in those with a one-locus-mismatched donor, and 10.3% (N=3) in those with a two- to three-loci-mismatched donor. In multivariate analysis the risk of graft rejection increased with increasing number of mismatches (1 mismatch HR 1.18, P= 0.86; 2 or more mismatches 8.58, P= 0.02; P for trend 0.03). The incidence of http://www3.interscience.wiley.com/cgi-bin/fulltext/118642867/main.html,ftx_abs - qt8#qt8grade II-IV GVHD was 39% in recipients with matched donors, 44% in one-locus mismatched and 50% in two- to three-loci mismatched. In multivariate Cox regression analyzes increasing HLA disparity was associated with increased risk of developing grade II-IV acute GVHD (1 mismatch HR 1.83, P= 0.04; 2 or more mismatches 2.44, P= 0.02; P for trend 0.01). The cumulative incidence of extensive cGVHD was 38%, 34% and 60% after transplantation with a matched donor, one-locus mismatched or two- to three-loci-mismatched, respectively. In the multivariate analyzes only a tendency towards increasing chronic GVHD with increasing HLA disparity was observed. Overall survival was 48%, 51% and 18%, in patients transplanted with matched donors, one-locus mismatched or two- to three-loci mismatched donors, respectively. Hundred-seventy-eight of the study patients died and no difference was observed in relapse or non-relapse related mortalities in relation to the degree of HLA disparity.

In a study from the DNA-Farber Cancer Institute, Ho et al. reported on the impact of HLA-C mismatch in a cohort of 111 patients transplanted with PBSC from unrelated donors after non-myeloablative conditioning with busulfan (3.2 mg/kg) and fludarabine (120 mg/m^2) (10). GVHD prophylaxis included cyclosporine/prednisone-based regimens and tacrolimus/mini-methotrexate-based regimens. Seventy-eight of the patients were 10/10 matched (HLA-A, B, C, DRB1 and DQB1), 21 were mismatched at a single HLA-C locus, three were double mismatched at HLA-C, and nine were mismatched at HLA-C plus another locus. Mismatching at HLA-C did not compromise engraftment, with median neutrophil and platelet nadir times being similar in all groups. Graft rejection only occurred in two patients in the mismatch group as compared to one patient in the matched group. Patients with HLA-C mismatch had higher incidence of grade II-IV and III-IV acute GVHD as compared to 10/10 matched patients (grade II-IV 42% vs 26%, P=0.04; grade III-IV 33% vs 12%, P=0.01). There was no difference in chronic GVHD between the two groups. Non-relapse mortality was significantly higher in the HLA-C mismatched patients (48% vs. 16%, P=0.0001), while a trend was observed toward higher relapse mortality in the matched patients (55% vs 35%, P=0.09). Overall survival at 2 years was significantly lower in mismatched patients (30% vs 51%, P=0.008) and presence of HLA-C mismatch was an independent risk factor for death (HR 1.85, *P*=0.04).

Ogawa et al. (21) reported a series of 26 patients transplanted with PBSC from haploidentical donors with 2-3 HLA antigen mismatches in the graft versus host vector. The conditioning regimen consisted of fludarabine (180 mg/m^2), busulfan (8 mg/kg) and ATG (8 mg/kg). Both to prevent ATG associated anaphylaxis and GVHD, methylprednisolone was administered from day -4 and tapered from day +15 to +30. Tacrolimus was started on day -1 (I.V. infusion 0.02 mg/kg; oral dose 0.08 mg/kg; target blood concentration 10–15 ng/mL) and tapered from day +30. All but one patient engrafted, and ten out of 25 evaluable patients developed acute GVHD (grade I, N=5; grade II, N=5). Out 20 patients evaluable, five developed extensive chronic GVHD. Four patients died of treatment related mortality and the 3-year relapse rate was 27.1% for patients in CR at time of transplantation and 29.8% in for patients not in CR. Overall survival was 55%.

In a recent study of 274 patients with AML the cohort included 34 who were transplanted with HLA mismatched donors (22). All patients were treated with PBSC after non-myeloablative conditioning (fludarabine 90 mg/m^2 and 2 Gy TBI) and calcineurin/MMF based immunosuppression. Of the 12 patients who experienced graft rejection only one was transplanted with a HLA mismatched donor. Rates of acute GVHD were higher in patients with HLA mismatched unrelated grafts as compared to patients with matched related or unrelated grafts (grade II, 50% vs 28% and 43%, respectively; grade III-IV, 24% vs 12% and 12%), while no difference was observed in chronic GVHD. In the multivariate analysis, increased HLA disparity between patient and donor was associated with increased risk of non-relapse mortality (HLA matched related, HR 1.00; HLA matched unrelated, HR 1.86; HLA-mismatched unrelated 3.91; P=0.003). Although HLA-mismatched unrelated recipients seemed to have less 5-year relapse/progression than HLA-matched related or unrelated recipients (25% vs 47% and 42%, respectively), these differences were not statistically significant. Furthermore, patients with HLA-mismatched unrelated donors had a slightly worse 5-year OS than patients with HLA-matched related or unrelated donors (22% *v* 37% and 33%, respectively; P = 0.37).

The locus-specific effect of HLA-mismatch was shown in a recent NMDP analysis of unrelated PBSC [Woolfrey, unpublished]. Among the subset of 616 patients given reduced intensity or nonmyeloablative conditioning, mismatching of HLA-C antigen (n=65) was associated with an increase in the risk of mortality compared to 8/8 matches (RR 1.40 [1.01-1.95], p=0.04). In contrast,

other 7/8 (non-C) antigen mismatched pairs and 7/8 allele mismatched pairs did not have statistically higher mortality than 8/8 matched pairs in either myeloablative or nonmyeloablative groups.

Although these studies are heterogeneous in all aspects of their design, collectively they demonstrate that sustained engraftment is achievable using reduced intensity or non-myeloablative conditioning. However, incidences of treatment related mortality and GVHD remain high.

3C. FHCRC Protocol 1591: Campath® [Alemtuzumab] Dose Escalation, Low-Dose TBI and Fludarabine Followed by HLA Class I Mismatched Donor Stem Cell Transplantation for Patients with Hematologic Malignancies - A Multi-Center Trial (11)

The purpose of this protocol was to test the feasibility of extending allogeneic HCT after non-myeloablative conditioning (fludarabine 90 mg/m^2 and 2 Gy TBI) to include donor grafts from HLA class I mismatched related or unrelated donors. Immunosuppression consisted CSP (5 mg/kg b.i.d.) from day -3 with taper from +180 to day +365 and MMF (15 mg/kg t.i.d.) from day 0 with taper from day +100 (11% weekly for eight weeks). Fifty-nine patients were enrolled on the protocol. All patients received PBSC from either a related (N=5) or unrelated donor (N=54). All recipient/donor pairs were mismatched for at least one HLA class I locus (A, B or C) at the antigen level, except for a single unrelated pair that had two mismatches at the allele level. In the subset of unrelated pairs, 10 had an additional class I allele mismatch, while no additional allele level mismatches were observed in related recipient/donor pairs. The study design included a provision for the addition of alemtuzumab to CSP and MMF, if the rejection rate exceeded 20%. However, all patients were transplanted without alemtuzumab, as the sustained engraftment rate was 95%. Only 43 patients were evaluable for rejection because 4 died less than 30 days after transplantation (2 of treatment related toxicity and 2 of disease progression), 8 recipient/donor pairs were mismatched in the GVHD vector only and 4 received planned tandem auto-allo transplants. The median neutrophil and platelet nadirs were 100 cells/μL (range 0-860 cell/μL) and 23x10^3 /μL (range 4-110x10^3 /μL), respectively. Ninety-one percent of the patients developed neutropenia for median of 9 days (range 0-33), while 38% of the patients developed thrombocytopenia for a median of 0 days (0-23). The cumulative incidences of grade II-IV acute, grade III-IV acute and extensive chronic GVHD were 69%, 26% and 41%, respectively (figures 1A-B). The cumulative probabilities of non-relapse mortality were 22% at day 100 and 36% at 1 year (figure 2A). Twenty-six patients died of non-relapse mortality with the most common causes being infection with (N=8) or without GVHD (N=4) (Table 1). Two-year overall and progression-free survivals were 29% and 28%, respectively (figure 2B). In summary, protocol 1591 demonstrated that sustained engraftment of HLA class I single antigen mismatched donor grafts is achievable with non-myoablative conditioning. However, cumulative incidences of non-relapse mortality and GVHD were high, and further studies addressing these issues are warranted.

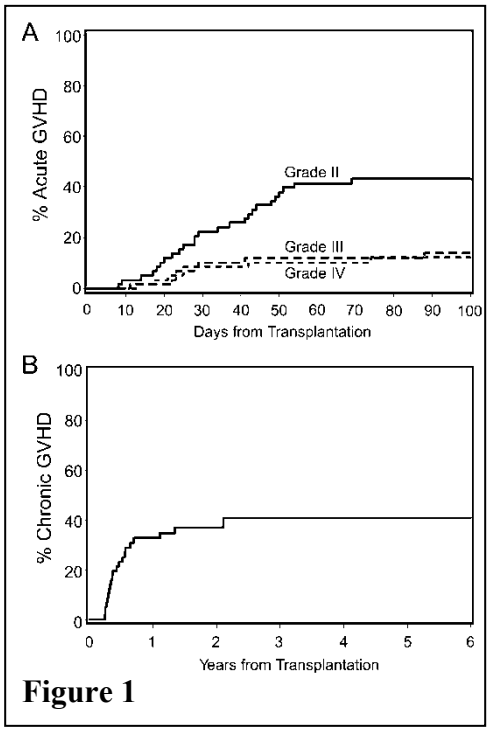

Figure 1

Table 1. Causes of non-relapse mortality in 26 patients

Diagnose	Within the 1st year after HCT, no.	More the 1st year after HCT, no.
Infection with GVHD	6	2
Infection without GVHD	2	2
GVHD*	3	1
Multiorgan failure	3	0
Secondary AML	1	0
Diffuse alveolar damage, ARDS	2	0
Cardiac failure	2	1
Leukoencephalopathy	1	0

*Includes 2 patients with bronchiolitis obliterans

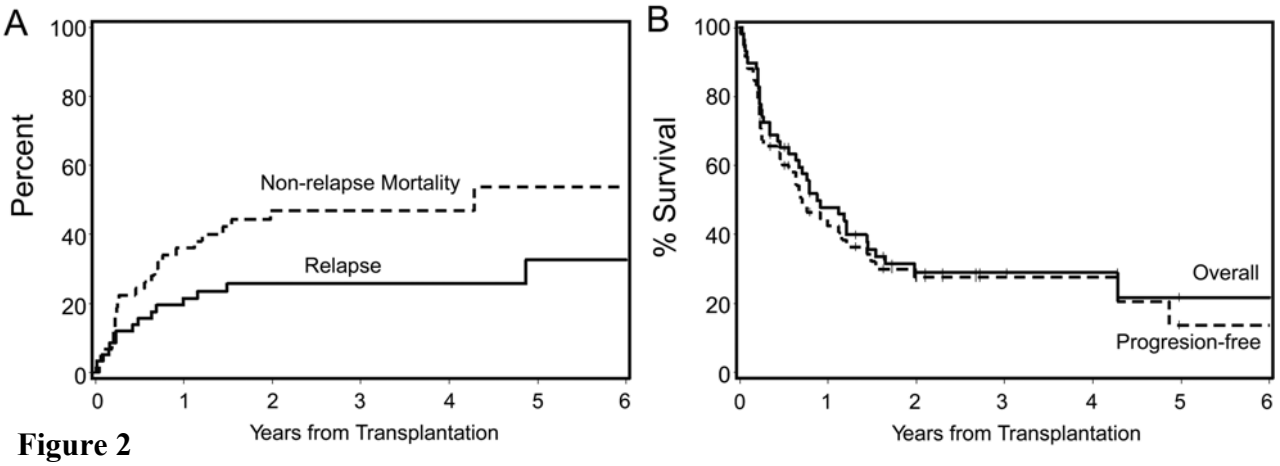

Figure 2

3D. Relationship between GVHD and graft versus tumor effects

We analyzed GVT effects in 322 patients with hematological malignancies given grafts from HLA-matched related (n=192) or unrelated donors (n=130) (23). Two-hundred and twenty-one patients had measurable disease at HCT and 126 of them (57%) achieved partial (n=28) or complete (n=98) remissions. In multivariate analysis, there was a trend for a higher probability of achieving complete remissions in patients with chronic extensive GVHD (p=0.07). One hundred and eight patients (34%) have relapsed or progressed. In multivariate analysis, grade II-IV acute GVHD had no statistically significant impact on the risk of relapse/progression, but was associated with increased risk of non-relapse mortality and decreased probability of progression-free survival. Conversely, extensive chronic GVHD was associated with decreased risk of relapse/progression (p=0.006) and improved probability of progression-free survival (p=0.003). These data suggest that outcomes of nonmyeloablative conditioning and unrelated HCT could be improved by more intensive post-grafting immunosuppression aimed at suppressing acute GVHD, while allowing chronic GVHD to occur

3E. Postgrafting immunosuppression

1. CSP and MMF

CSP and MMF will be used in the current protocol as this combination enabled sustained engraftment in protocol 1591. However, the exact schedule for their administration will be adjusted due to experiences derived from FHCRC protocols 1463, 1641, 1668 and 1938. All patients enrolled in these protocols were transplanted with unrelated donors after non-myeloablative conditioning with fludarabine 90mg/m^2 and 2 Gy TBI. Three patients with CML on protocol 1668 received 3 Gy of TBI. Protocol 1938, which is a randomized phase II study investigating MMF, tacrolimus and sirolimus, will be discussed in section 3E.2.b.

a) FHCRC Protocol 1463(2) and 1641(24)

Eighty-nine patients were enrolled on protocol 1463. All patients were transplanted with PBSC or bone marrow from fully matched unrelated donors (10/10 HLA-A, -B, -C, -DR, and –DQ). Post-grafting immunosuppression consisted of MMF and CSP. MMF was given b.i.d. (15 mg/kg) from day 0 to +40 and then tapered off by day +96, while CSP that was given b.i.d. (6.25 mg/kg) from day –3 to +100 and tapered off by day +180.The cumulative incidence of grade II-IV acute GVHD was 52% and 37% for extensive chronic GVHD. The probabilities of 1-year overall survival and progression free survival were 52% and 38%, respectively. Data from protocol 1463 demonstrated significant differences in outcome between recipients of PBSC or bone marrow. Although the sustained engraftment rate in the whole cohort only was 79%, engraftment was significantly higher in recipients of PBSC than recipients of bone marrow (85% vs 45%, P=0.007). Progression free survival was also higher in recipients of PBSC (44% vs. 17%, P=0.02), despite an increased cumulative incidence of grade III-IV acute GVHD (11% vs. 0%, P=0.05). Pharmacokinetic studies of MMF, demonstrated that the halflife of its active metabolite was 3 hours, suggesting that better immunosuppression could be obtained by more frequent dosing of MMF.

Based on the data derived from protocol 1463 protocol 1641 was developed. It was essentially the same as the original, however to ensure engraftment and reduce acute GVHD, the MMF dosing schedule was changed to t.i.d. and all patients were transplanted with PBSC. Ninety-nine patients were enrolled on protocol 1641. With three daily doses of MMF the median day +28 T-cell chimerism increased to 92% compared to 75% (P=0.02) and sustained engraftment was 95%. Cumulative incidences of acute (52%) and chronic (40%) GVHD were similar to protocol 1463. One-year overall survival, progression-free survival, relapse/progression, and non-relapse mortality were 64%, 54%, 27%, and 19%, respectively.

In a combined analysis of PBSC recipients in both protocols (N=174) the diagnoses of CML and MDS/MPS were associated with a greater risk of graft rejection (P=0.0006) relative to all other diagnoses. Furthermore the 1-year non-relapse and relapse related mortalities were 25% and 35%, respectively for MDS/MPS patients, which translated into an inferior 1-year overall survival (40% vs. 60-80% for leukemia, lymphoma and multiple myeloma). One-year progression free survival was also inferior for both MDS/MPS and CML patients (30% vs. 55-60% for leukemia, lymphoma and multiple myeloma). The reason for the poor progression free survival for MDS/MPS patients was high relapse and nonrelapse mortality rates and for CML patients, disease progression after graft rejection.

b) FHCRC Protocol 1668(25)

Several studies have suggested that CSP prevented activation induced death of T-cells, and thus potentially delayed eradication of alloreactive donor T-cells, hereby preventing tolerance induction (26,27). Conversely, antimetabolites such as MMF could delete autoreactive T cells by inducing apoptosis (28, 29). The goal of protocol 1668 was to reduce the incidence of GVHD by translating these experimental findings into the clinical setting. The period of CSP administration was shortened (5mg/kg b.i.d. from day -3 to +80) while the duration of MMF was prolonged (15mg/kg t.i.d from day 0 to +30, then b.i.d. until day +150 and taper to day 180). Seventy-one patients were enrolled, all transplanted with PBSC from fully matched unrelated donors. Sustained engraftment was observed in 96%, with lower overall survival and progressionfree survival as compared to a cohort 103 of historical controls (overall survival: 55% vs. 68%, P=0.05; progression free survival 47% vs. 56%, P=0.05 (adjusted for pre-transplant risk factors)). One-year relapse incidence was similar between the two cohorts (23% vs 26%). However, nonrelapse mortality was significantly higher in the current protocol (29% vs 18%, P=0.02). The patients who were without grade II-IV acute GVHD at day+80, and therefore had their CSP stopped, had significantly higher risk of experiencing non-relapse mortality (HR 10.1, P<0.0001), as compared to patients who were continued on CSP due to GVHD. Current and historical non-relapse mortalities prior to CSP cessation at day +80 were comparable. Furthermore cumulative incidences of grade II-IV and III-IV acute GVHD were 77% and 26% among current patients versus 52% and 15% among historical patients, respectively. Among the current patients, 7 experienced grade III-IV acute GVHD, of which 4 were in immediate relation to cessation of CSP administration (3 at day +80 and 1 due to disease progression). The cumulative incidence of chronic GVHD in the current protocol was 45%, and similar to the historical cohort. In summary, we observed that prolonging MMF and truncating CSP increased the cumulative incidence of acute GVHD instead of reducing it. Thus, suggesting that administration of a calcineurin inhibitor for at least 6 months is needed to establish graft-host-tolerance (25).

2. <u>Sirolimus (Rapamune®)</u>

a) 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) (30). 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 $CD4^+CD25^{high}$ (31)[Gao1]. Evidence points to that 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 [Frank1] (32,33). The preferential

expansion of T_{reg} is attenuated when sirolimus is used in combination with CSP[Gao1]. 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 (34)[Hanash1] (35).

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 (36).

ii) Viral amplification. Inhibition of mTOR may also have effects on viral amplification, as CMV specifically upregulates the mTOR pathway during replication (37). In allogeneic HCT and solid organ transplantation lower risk of CMV activation has been reported in patients treated with sirolimus (13,38).

iii) Antineoplastic effects. The mTOR signaling pathway is often constitutively activated in various human cancers. The efficacy of sirolimus as an antiangiogenetic antineoplastic agent has been demonstrated in several experimental cancer models (39-41). 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) (42).

b) Sirolimus for acute GVHD prophylaxis. Four clinical trials with sirolimus have been published from the Dana-Farber Cancer Institute (Table 2) ((12), (13),(14),(15)). 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 a 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 (MAC vs RIC (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 2).

Table 2. Summary of clinical trials

	Antinet al. (12)1	Cutler et al. (13)	Cutler et al. (14)	Alyea et al. (15)	FHCRC 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.

In a small study of 15 patients receiving primarily PBSC from 9 unrelated and 6 related fully HLA matched donors after RIC (FLAMSA-RIC), the GVHD prevention was comprised of a combination of sirolimus (4 mg daily starting on day -1, with a target concentration of 5-10 ng/ml and taper at day +60 to +90) and MMF (1000 mg administered 6-12 hrs after transplantation, hereafter 2000 mg daily with reduction and termination at day +50) (16). Although sirolimus was started as late as one day prior to the transplant, satisfactory GVHD control was obtained, with only two patients experiencing acute GVHD (1 grade II and one grade IV) and three experiencing chronic GVHD.

In a recent study by Snyder et al. (17), 23 patients with myelofibrosis were transplanted using a primarily fludarabine and melphalane based RIC regimen. The first nine patients received CSP/MMF based immunosuppression, while the last 14 received a combination of sirolimus and tacrolimus analogous to the studies by Antin et al. and Cutler et al. All patients were transplanted with PBSC and 15 out of 23 with grafts from unrelated donors (7 out of 9 in the CSP/MMF group and 8 out of 14 in sirolimus/tacrolimus group). 13 out of the 15 patients transplanted with unrelated donors received additional immunosuppression with MTX 5 mg/m^2 on day +1, +3 and +6 (6 in the CSP/MMF group and 7 in sirolimus/tacrolimus group). Sixteen of the 23 patients experienced acute GVHD with no significant difference between the CSP/MMF and sirolimus/tacrolimus groups. However in the subset of patients who developed grade III-IV acute GVHD (N=5) the

cumulative incidence was significantly higher in the CSP/MMF group as compared to the sirolimus/tacrolimus group (60% vs. 10%, P=0.01). No patients in the sirolimus/tacrolimus group succumbed to treatment related causes (day 100 treatment related mortality 0% vs 33%, P=0.02), which translated into a superior overall survival at 2 years (93% vs 56%, P=0.05).

FHCRC protocol 1938 is a randomized 3-arm phase II study comparing the efficacy of different combinations of MMF, tacrolimus and sirolimus in preventing acute GVHD (Figure 3). By May 2010 183 patients had been accrued (arm #1 (control), N=60; arm #2, N=61: arm #3, N=61). Most were transplanted with PBSC from fully matched unrelated donors (single HLA class I mismatch in 9%) after non-myeloablative conditioning (90 mg/m^2 and TBI 200 cGy). Sirolimus was administered in combination with MMF and tacrolimus, from day -3 to +80, with a daily dose of 2 mg and a target concentration of 3-12 ng/ml (figure 3). The cumulative incidence of grade II-IV acute GVHD was lower in the sirolimus arm as compared to the tacrolimus/MMF only arms, although the difference was only significant when compared to the control arm (figure 4A). Dividing acute GVHD into separate grades, the addition of sirolimus lowered the cumulative incidence of grade II acute GVHD as compared to the tacrolimus/MMF only arms (figure 4A). No difference between treatment arms was observed for grade III-IV acute GVHD (arm #1 12%; arm #2 13%; arm #3 11%) or chronic GVHD (arm #1 43%; arm #2 42%; arm #3 46%). Furthermore no differences between treatment arms were observed for nonrelapse mortality and relapse/progression (figure 4C-D). When the results of protocol 1938 were compared to a historical

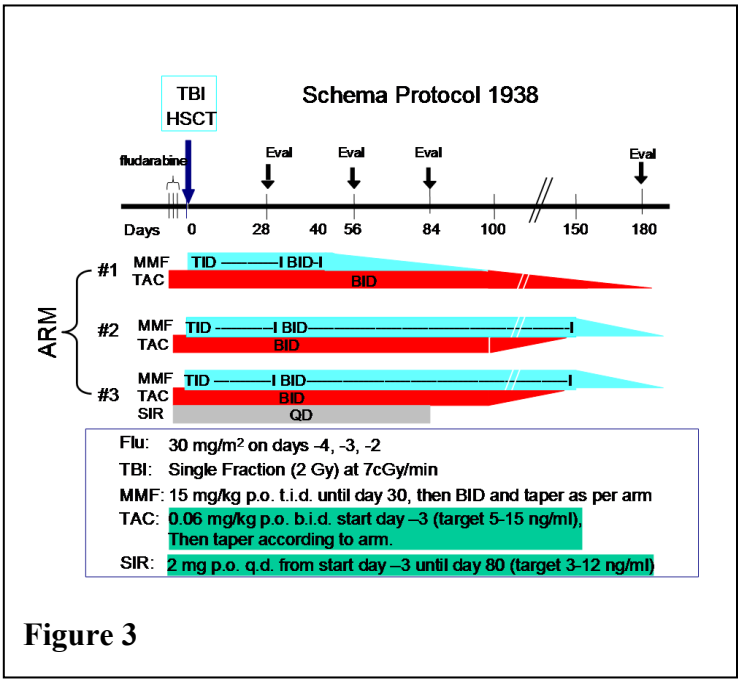

Figure 3

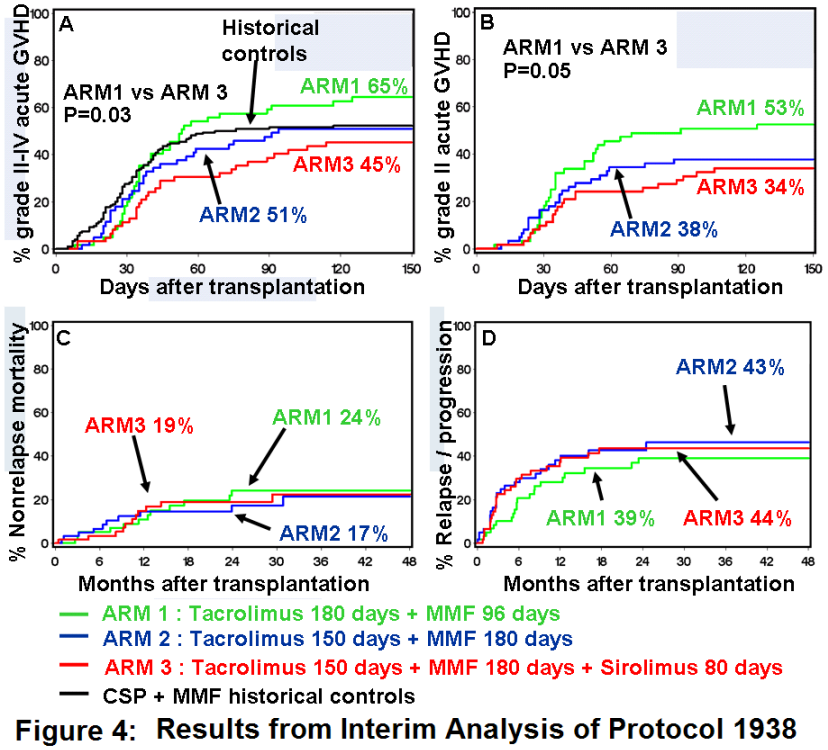

Figure 4: Results from Interim Analysis of Protocol 1938

cohort 174 unrelated non-myeloablative transplants treated with a combination of CSP and MMF, no difference was observed in the cumulative incidences of nonrelapse mortality, relapse/progression, and acute (figure 4A) and chronic GVHD as compared to the tacrolimus/MMF only arms. Thus, suggesting that the substitution of CSP for tacrolimus did not entail additional GVHD control.

All patients in the four studies from Dana-Farber Cancer Institute and in FHCRC protocol 1938 engrafted. Median times to neutrophil and platelet engraftment were between 13-18 days and 13-29, respectively.

There has been a concern that sirolimus treatment added to the toxicity of the calcineurin inhibitors. In a retrospective analysis by Cutler et al. (43) from 2005, post transplant toxicities were compared between 111 and 216 patients treated with sirolimus and non-sirolimus based immunosuppression. Patients in the sirolimus group had a significantly higher incidence of thrombotic microangiopathy post-transplant (10.8% vs 4.2%, P=0.03). However, in the trial by Cutler et al. from 2007(14) the incidence was not increased, and in FHCRC protocol 1938 there was no difference in the incidence of toxicities across the three treatment arms.

c) The rationale for the use of sirolimus in combination with CSP and MMF for acute GVHD prophylaxis after related or unrelated donor HCT and nonmyeloablative conditioning.

The rationale for the current proposal is that sirolimus induced mTOR inhibition, provides immunosuppression at a different point in T-cell activation than MMF and the calcineurin inhibitors leading to synergistic effects(30,44-47). Data from FHCRC protocol 1938 have already demonstrated that the addition of sirolimus elicits improved control of acute GVHD, as compared to the combination of MMF and a calcineurin inhibitor alone. Furthermore, due to the unique effect on mTOR, which is involved in numerous cellular processes, the addition of sirolimus may also have an adventitious influence on CMV activation and tumor control.

3F. The use of 3 Gy TBI in place of 2 Gy TBI for patients at higher risk of rejection.

We have shown that the risk of rejection increases for certain diseases (MDS, MPD and CML), as well as for those patients previously transplanted with either syngeneic or allogeneic stem cells. Thus, for these scenarios and while maintaining a non-myeloablative platform, the following patients will receive 3 Gy (rather than 2 Gy) TBI.

4. Proposal

The goal of the current trial is to determine whether the incidence of acute GVHD can be reduced after non-myeloablative HLA class I or II mismatched donor HCT to less than the historical rate of 70% by adding sirolimus to CSP and MMF. A combination of CSP and MMF will be used, as this combination enabled sustained engraftment after non-myeloablative HLA class I mismatched donor HCT, without the need for additional alemtuzumab (FHCRC protocol 1591). As a result of insufficient GVHD prevention in protocol 1591, sirolimus will be added to the combination of CSP and MMF, as data from protocol 1938 demonstrated better GVHD control with this combination. The choice of CSP over tacrolimus is also derived from data from protocol 1938, which showed no significant difference in transplantation outcome, between patients treated with the tacrolimus and MMF combination compared to a historical cohort treated with CSP and MMF. Furthermore, the MMF scheme from protocol 1938 with administration three times daily for the first 30 days post-transplant, and then twice daily for the remaining period enabled engraftment

without increasing GVHD. To avoid the detrimental effect of CSP on the possible sirolimus induced selective expansion of T_{reg}, the duration of CSP treatment will be shorter than sirolimus. However due to development of grade III-IV acute GVHD coinciding with early termination of CSP in protocol 1668, CSP taper will first start at day 150 in the current protocol. The treatment schedule is outlined in figure 5 (page 20).

5. Primary Objective

To determine whether the incidence of acute GVHD grades II-IV can be reduced to less than the historical rate of 70% with the triple-immunosuppressant combination of CSP/MMF with sirolimus in HLA class I or class II mismatched related or unrelated donor HCT using nonmyeloablative conditioning. The evaluation will be carried out separately among class I and class II mismatched patients.

6. Secondary Objectives

- To evaluate the incidence of non-relapse mortality before day 100
- To evaluate the incidences of grades III-IV acute GVHD

7. Patient Selection

A. Inclusions

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

Ages ≤ 50 years of age 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 (>40% risk of TRM). This criterion can include patients with a HCT-CI score of ≥1 (**see Appendix Q**). Transplants should be approved for these inclusion criteria by the principal investigators at the collaborating centers and at FHCRC. All children < 12 years must be discussed with the FHCRC PI (Brenda Sandmaier, MD 206 667 4961) prior to registration.

Ages ≤ 50 years of age with chronic lymphocytic leukemia (CLL).

Ages ≤ 50 years of age with hematologic diseases treatable by allogeneic HCT who refuse a high-dose HCT. Transplants must be approved for these inclusion criteria by the principal investigators at the collaborating centers and at FHCRC.

The following diseases will be permitted although other diagnoses can be considered if approved by PCC or the participating institutions' patient review committees and the principal investigators.

- **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; 4) Patients with a diagnosis of CLL (or small lymphocytic lymphoma) that progresses to prolymphocytic leukemia (PLL); or 5) patients with T-cell CLL or PLL.
- **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)** – Patients must have <5% marrow blasts at time of transplant.
- **Waldenstrom's Macroglobulinemia –** must have failed 2 courses of therapy.

Patients with related or unrelated donors for whom the best available donor is:

a. Mismatched at antigen level for any single class I locus (HLA-A, -B, -C) ± an additional class I mismatch at the allele level

OR

mismatched at the allele level for any 2 class I loci (if typed at the molecular level).

OR

mismatched at the antigen or allele level for class II loci HLA-DRB1 and/or –DQB1. Must be matched for at least one DRB1 allele and one DQB1 allele

b. there is a likelihood of rapid disease progression while HLA typing and results of a preliminary search and the donor pool suggests that a 10/10 HLA-A, B, C, DRB1 and DQB1 matched donor will not be found

c. there is no HLA-A, -B or -C one locus allelic mismatched donor available

B. Exclusions

1. Patients for whom the best available donor is mismatched at both HLA class I and class II.
2. A positive cross-match exists between the donor and recipient.
3. Patients with rapidly progressive intermediate or high grade NHL
4. Patients with a diagnosis of CMML.
5. Patients with RAEB-2 who have not received myelosuppressive chemotherapy i.e. induction chemotherapy.

6. Presence of circulating leukemic blasts (in the peripheral blood) detected by standard pathology for patients with AML, ALL or CML.
7. Presence of ≥ 5% circulating leukemic blasts (in the peripheral blood) detected by standard pathology for patients with MDS/MPS
8. CNS involvement with disease refractory to intrathecal chemotherapy. For LP requirement see **Appendix N**.
9. Fertile men or women unwilling to use contraceptives during and for up to 12 months following treatment
10. Female patients who are pregnant or breast-feeding
11. HIV positive patients
12. Patients with active non-hematologic malignancies (except non-melanoma skin cancers) or those with non-hematologic 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.
13. Fungal infections with radiological progression after receipt of amphotericin B or active triazole for greater than 1 month
14. Patients with active bacterial or fungal infections unresponsive to medical therapy.
15. Organ Dysfunction.
    a. Cardiac ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of < 26%). Ejection fraction is required if the patient is > 50 years of age, or history of cardiac disease or anthracycline exposure. Patients with a shortening fraction < 26% may be enrolled if approved by a cardiologist.
    b. Pulmonary:
        i. corrected DLCO < 40%, TLC <40%, FEV1 <40% and/or receiving supplementary continuous oxygen. If unable to perform complete PFTs, patients will be excluded if their oxygen saturation is <95% with a formal six-minute walk test (ambulatory oximetry).
        ii. The FHCRC 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.
16. Patients with poorly controlled hypertension on multiple antihypertensives
17. Karnofsky scores < 60 (see appendix B) or Lansky Score <50 (see appendix C).
18. All patients receiving antifungal therapy voriconazole, posaconazole, or fluconazole must have sirolimus reduced according to the Standard Practice Antifungal Therapy Guidelines in Appendix E.
19. 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.

8. Donor Selection

A. Inclusions

1. Related or 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, OR
   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 –DQB.
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.
4. 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 donor should be excluded if any of the flow cytometric B and T cell cytotoxic cross match assays are positive**.
5. Only G-CSF mobilized PBSC only will be permitted as a HSC source on this protocol.

B. 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 locus 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

9. Informed Consent

Both patient and donor (and their parents/guardians) will meet with a physician to thoroughly discuss treatment recommendations regarding this protocol and alternative treatment options for the underlying disease. The physician will explicitly address the potential known risks associated with the use of fludarabine, low-dose TBI and immune suppressive drugs (CSP/MMF/sirolimus). Risks should be addressed as objectively as possible. Patients should be informed that they have advanced malignancy with life expectancy of months to no more than 1 or 2 years with conventional treatments, that they would unlikely benefit from autologous transplant and are at very high risk of early treatment related mortality from high dose transplants (if a high dose transplant would be applicable at all). For the related stem cell donor, the procedure for collecting peripheral mononuclear cells and toxicity of G-CSF treatment will be explained as well as the potential need

and risks for several time points of leukapheresis. A summary of the conference should be dictated for the medical record detailing what was covered. Informed consent from the patient will be obtained using forms approved by the Institutional Review Board (IRB) of the Fred Hutchinson Cancer Research Center or the performing center if the patient is treated in a collaborating institution.

10. Protocol Registration

FHCRC 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.

Collaborating institutions: Eligible patients will be identified by the principal investigators of the collaborating institutions who will register the patient with the FHCRC. Registration will include completion of the eligibility checklist/demographic form (Appendix L). This form will be faxed to the Trial Coordinator (206-667-5378). Questions regarding eligibility or protocol information should be directed to Brenda Sandmaier, MD (206-667-4961)

11. Plan of Treatment

A. Outline Treatment Plan (Figure 5)

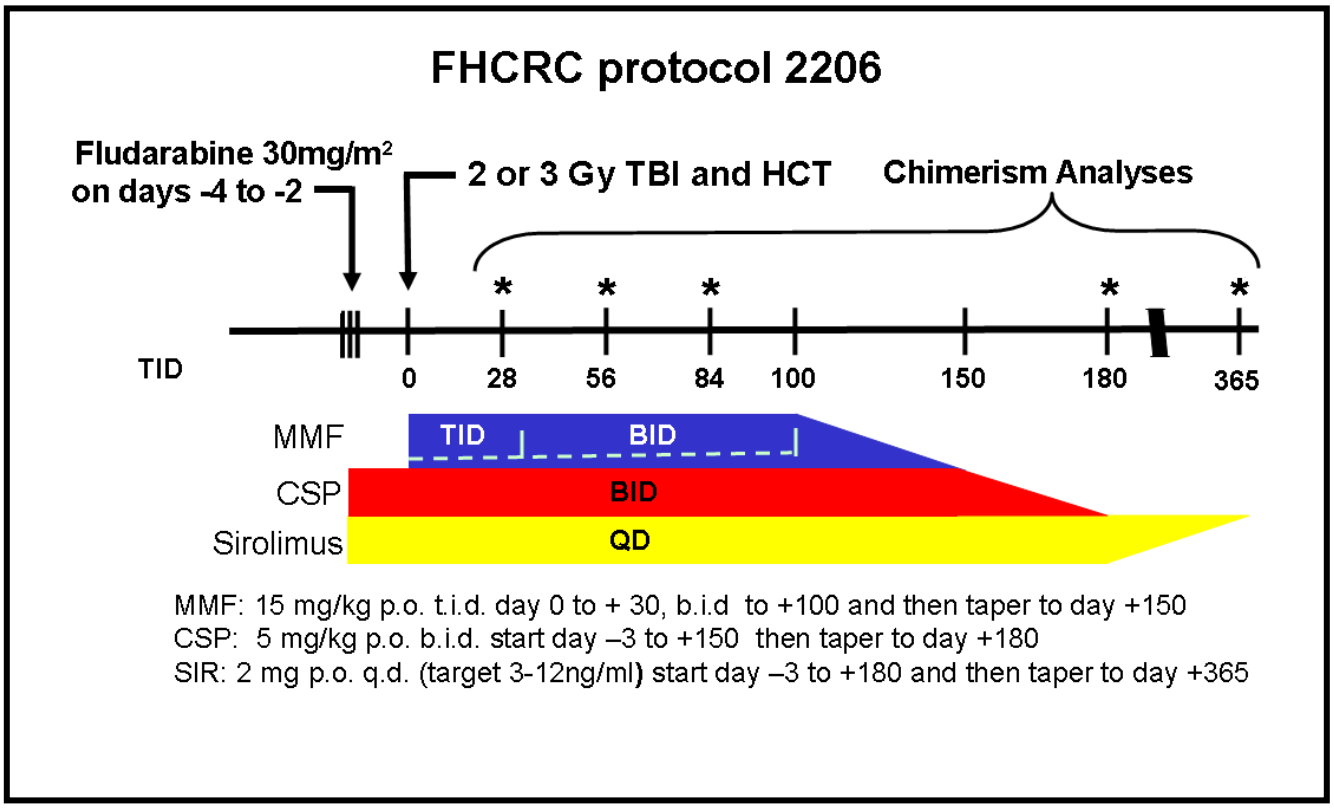

Figure 5. *, evaluation of T-cell chimerism; If T-cell chimerism is ≤ 50% on day +28, then repeat only day +56, +84, +180 and +365. If T-cell chimerism is > 50% on day +28, then repeat only day +84 and +365. Granulocyte (CD33+) chimerism on day +84 only. Natural Killer cell (CD56+) chimerism will be obtained on day +28. Bone Marrow chimerism will be obtained on day +84 and 365 (See Patients Post-transplant Evaluation For more detail)

Table 3: Conditioning and Immunosuppression Schedule

Day	-4	-3	-2	-1	0	30	100	150	180	365
fludarabine (30mg/m²/day)	▼	▼	▼							
TBI (Gy)					**2 or 3**					
PBSC transfusion					▼					
CSP (5mg/kg, PO q12 hr)		**Start**	⇨	⇨	⇨	⇨	⇨	**Taper[A]**	**Stop**	
MMF (15mg/kg, q8hrs)					**Start[B] q8hrs**	**q12hrs[C]**	**Taper[D]**	**Stop**		
Sirolimus (2mg QD)		**Start**	⇨	⇨	⇨	⇨	⇨	⇨	**Taper**	**Stop[E]**

A. CSP should only be tapered on day 180 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. Dosing schedule is changed from 15mg/kg T.I.D to 15mg/kg B.I.D.
D. The MMF taper will be a weekly dose reduction of approximately 11-12%.
E. The continuation of sirolimus for patients with severe acute GVHD is at the discretion of the attending physician.

B. HCT

Please refer to section 11.I.1 for information regarding the collection and infusion of related and unrelated donors.

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 (or the interval in which a cycle of standard chemotherapy would be administered in a non-transplant setting) prior to initiating the nonmyeloablative transplant conditioning (see exclusion criteria pages 16-17). 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.), 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 other all diagnoses.

F. Pre-transplant tyrosine kinase inhibitors (imatinib mesylate, dasatinib, nilotinib etc.).

1. All patients with the diagnosis of CML or Ph+ ALL may continue treatment with imatinib mesylate, dasatinib, nilotinib 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, dasatinib, or nilotinib 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, dasatinib, or nilotinib on the CNS are not known.

G. Conditioning Regimen (Refer to Figure 5 and Table 3)

1. Days –4, -3, and –2: Fludarabine 30mg/m^2/day IV, administered over 30 min.

2. Day 0: TBI 2 or 3 Gy at 6-7cGy/min from linear accelerator (Appendix V) followed by HCT. TBI to be administered between 10:00 a.m. and 2:00 p.m. to avoid proximity to MMF administration.

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 (200 vs 300 cGy) with PI.

H. Immunosuppression

a. Cyclosporine

1. Starting dose:
   i. **Adult dose**: CSP is given based on adjusted body weight, at 5.0 mg/kg PO q12 hours from day –3. If there is nausea and vomiting at anytime 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.
   ii. **Pediatric dose**: Due to the variable and increased metabolism in children, CSP will be started intravenously on day –3 at the doses listed below.
      i. Age ≤6 years old: 1.6 mg/kg IV q8 hours
      ii. Age >6 years old: 2.0 mg/kg IV q12 hours
   iii. Sirolimus should be given at least 4 hours after an oral dose of CSP as concurrent administration leads to elevation of sirolimus levels.
2. Cyclosporine discontinuation:
   i. In the absence of acute or chronic GVHD, CSP is tapered at day 150 over 30 days (to be completed on Day +180).
   ii. 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 (increase in serum or urine paraprotein by ≥25%) 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 therapy are summarized below (section 11.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.

Table 4: **CSP Dose Adjustment**

	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

   ii. Do not exceed cyclosporine levels > 350 ng/mL to reduce risk of sirolimus toxicity.
   iii. CSP Monitoring: CSP determinations should be performed on a twice weekly basis for the first month and then weekly until day +100 unless high levels are detected (i.e., >400ng/ml), or toxicity is suspected in which case more frequent monitoring will be performed as clinically indicated. Routine monitoring of CSP will not be required for patients on a CSP taper unless clinically indicated.
   iv. 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 4. There are two methods for calculating CSP levels. Table 4 provides desired levels for specific methods. To avoid inadequate immune suppression, dose reductions should be conservative. Therapeutic levels of CSP should be maintained.
   v. After day +28, typical serum CSP transplant levels for related or unrelated HCT will be targeted.
   vi. Dose reductions should only be made if CSP toxicity is present and/or levels exceed values provided in Table 4. Dose reductions for high levels without toxicity should be conservative e.g. 25%, to avoid inadequate immunosuppression.
   vii. If there is nausea and vomiting at anytime 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.
   viii. Oral Sandimmune may be substituted for oral Neoral.

ix. Patients requiring hemodialysis should be have CSP levels maintained in the high therapeutic range (Table 4).
x. Drugs that may affect CSP levels are (table 5):

Table 5

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

***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: MMF will be given daily at 15 mg/kg Q8 hours until **day +30**, and then in the absence of GVHD, the dose will be changed to 15 mg/kg Q12 hours until **day**

100. In the absence of GVHD, MMF will be tapered at **day 100** by 11-12% per week and discontinued at day +150.

3. 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).
4. Guidelines for MMF dose adjustment due to drug toxicity:
   i. If in the clinical judgment of the investigator or Attending 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 21 post-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

1. Sirolimus dosing:
   i. Sirolimus should 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 (37,48,49). 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) (48). 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 (50).

ii. **Patients with BSA > 1.5:** Sirolimus will be started on day –3 at 2.0 mg every day orally through day 180. In the absence of GVHD, Sirolimus should be tapered at day +180 with an adapted dose reduction and discontinued 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:** For children and 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.

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.

2. Dosing will be adjusted to maintain a target blood level of 3-12 ng/mL until day 80. 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 a week or as clinically indicated. 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 R).

3. 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.

4. If there is evidence of disease progression and no evidence of GVHD, patients will stop sirolimus and MMF without a taper per Attending discretion. Taper CSP within 2 weeks, and be observed for 1-2 weeks off of immunosuppression. If no GVHD occurs, patients with progressive disease will be offered chemotherapy (either standard or on a research trial). If appropriate, DLI (other institutional protocols for DLI) will be offered either in place of or after chemotherapy, depending upon disease and tempo of relapse/progression.

5. Patients who are experiencing either suspected or documented fungal infection, alternative therapy should be administered whenever possible. If voriconazole, posaconazole, or fluconazole are deemed necessary, sirolimus dosing reductions must be followed according to the Standard Practice Antifungal Therapy Guidelines in Appendix E due to contraindications.

6. Severe neutropenia or thrombocytopenia. The combination of sirolimus and cyclosporine interaction is that there is an increase risk of sirolimus toxicity such as anemia, diarrhea, hypokalemia, and 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 QD and dose increased to 2 mg/qd as long as severe hematopoietic toxicity does not occur

I. Collection and Infusion of Donor cells

1. G-CSF Administration to Donors and PBSC collection

Related Donor (RD):
All related donors will receive G-CSF 16μg/kg SQ q.d. for 5 consecutive days from day –4 to day 0. Doses will be administered before 10:00 a.m. and after 4:00 p.m. each day in the Ambulatory Clinic. The schedule of G-CSF administration and PBSC collections can only be ascertained once day 0 is identified. Once a treatment schedule has been fixed and the schedule of G-CSF administration and PBSC collections is made, the plan has to be confirmed with the personnel in the apheresis room. Day 0 should be fixed on a Tuesday – Thursday. PBSC will be collected in the afternoon of day –1, stored in the refrigerator at 4°C overnight. A second collection will be performed the following afternoon and both collections will be infused on day 0. The physician responsible for HCT collection will obtain informed consent from the donor.
Unrelated Donor (URD):
Timing of PBSC collection will be prearranged through the National Marrow Donor Program (NMDP) or other international donor centers in the case of an unrelated donor. Day 0 should be fixed on a Monday – Thursday when possible. G-CSF will be administered by subcutaneous injection to the donors starting 5 days prior to the day 0 according to the current NDMP protocol. Donors will receive approximately 10μg/kg of G-CSF each day of mobilization. A 12 liter apheresis will be obtained on day –1 and possibly on day 0 for a total of 12 to 24 liters of apheresis collection that will be infused on day 0.

Table 6 Treatment Schema for Related (RD) and Unrelated Donors (URD)

Day	-5	-4	-3	-2	-1	0
G-CSF SQ **(~10μg/kg for URD)** **(16μg/kg for RD)**	X	X X	X X	X X	X X	[X] X
PBSC collection					X	X

a. Immunophenotyping of the PBSC graft**.**
Immunophenotyping of the PBSC product for the Seattle patients will be performed by the CTL 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.

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 the donors will be frozen according to standard cryopreservation for DLI. Donor PBSC products will be frozen in an aliquot of 3.0×10^7 CD3+ cells/kg.

2. HCT infusion: All patients will receive unmodified 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 D. 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 (52). 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, nilotinib, etc.) for Ph (+) CML or A.L.L. 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.
   Patients < 18 years: Imatinib at 340 mg/m^2 orally each day, not to exceed 600 mg per day.

2. **Dasatinib (Sprycel)**: The suggested starting dose is:
   Patients ≥ 18 years: Dasatinib at 70 mg orally BID (twice per day).
   Patients < 18 years: who are potential candidates for BCR/ABL tyrosine kinase inhibitor therapy (other than Imatinib or Nilotinib) after HCT should be presented to PCC for discussion, and PI approval.

3. **Nilotinib (Tasigna)**: the suggested starting dose is:
   Patients ≥ 18 years: Nilotinib at 400 mg orally BID (twice per day).
   Patients < 18 years: Nilotinib at 230 mg/m^2 orally BID, not to exceed 400 mg po BID.

   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. **<u>If the bone marrow cellularity is < 10%, and blasts < 5%,</u>** 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. <u>If bone marrow cellularity is >10% and/or blasts >5</u>**<u>%, the tyrosine kinase inhibitor therapy can be increased or other therapy considered (see section 11.O.6.e).</u>**

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. **<u>Definition of mixed donor/host chimerism, engraftment, graft failure and rejection</u>**. 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 to 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). 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 (B.Sandmaier) of the protocol and the guideline in Appendix H 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:

6. **a. Discontinuation of immunosuppression:** This should be considered the first therapeutic maneuver. If there is no GVHD, MMF and sirolimus are to be stopped. CSP should be tapered over 2 weeks. Bone marrow aspirate and blood chimerism studies will be performed when off immunosuppression after 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. DLI will not be offered for disease progression or relapse on this protocol. 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 other 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.

d. CML or Ph(+) A.L.L. patients with Persistent or Increased Minimal Residual Disease: At day +84 or beyond, if the patient has persistent or increased MRD disease, dose escalation of BCR/ABL tyrosine kinase inhibitor therapy or DLI should be considered

e. CML and Ph (+) A.L.L. patients with Relapse and Disease Progression: See above sections for withdrawal of immunosuppression based on treatment arm. If there is no response to stopping immunosuppression and there is no GVHD, dose escalation of BCR/ABL tyrosine kinase inhibitor therapy and or DLI should be considered). (Suggested doses for adults are Imatinib to 800 mg QD or dasatinib to 90 mg BID).

12. Assessment of Disease Responses

The initial anti-tumor effect of allogeneic 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 H. Standard response criteria specific to other diseases will be used in assessing disease response for other patients on study.

13. 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 or Lansky score, HCT CI score (**Appendix Q**) 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. If unable to perform complete PFTs with a DLCO, patients will be evaluated with a formal six-minute walk test (ambulatory oximetry).
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 **Appendix N** 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.

Additionally, see the following tables (Tables 7, 8, 9, 10) for disease specific pre-transplant evaluations.

Table 7: 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. See Tables 11 and 12 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 FHCRC patients only*
PCR for bcr/abl, p.190 and p.210 breakpoints - **see comment*	Clinical	**Ph (+) ALL only- reflexive testing for FHCRC 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 8: Disease-Specific Pre-Transplant Evaluations for AML and MDS/MPD
Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected within **21 days** of treatment. See Tables 11 and 12 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 only*
Peripheral Blood		
Storage for chimerism analysis	Clinical	

Table 9: 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. See Tables 11 and 12 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	
ZAP – 70 by flow cytometry- **see comment*	Clinical	**CLL only– for patients not in CR*
Imaging		
CT of chest, abdomen, pelvis (neck if indicated)	Clinical	

Table 10: 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 11 and 12 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*

B. Patient Post-transplant Evaluation

1. See Table 11 for disease specific post-transplant evaluation on Day +28, 56, 84, etc. This is a recommended evaluation schedule.

Additionally, include the following for all diseases:

2. History and physical exam to assess Karnofsky performance status and GVHD weekly until day +84, thereafter monthly or as indicated. If GVHD develops refer to Toxicity section.
3. CBC three times a week, or more often if clinically indicated, from day 0 until day +28, and twice weekly until 2 months post-transplant or later if clinically indicated
4. Cyclosporine trough levels on day "0" and then twice a week until taper begins. Weekly thereafter if levels are stable.
5. Sirolimus trough levels on day "0" and then twice a week for the first month and weekly thereafter to maintain therapeutic serum levels.
6. Chem 1(Na+, K+, Cl_, Bun, Cr, glucose) and chem 3 (Mg+2, Ca+2) 3x per week until CSP taper begins.
7. a) Serum triglyceride levels (fasting) every two weeks post transplant until Day +56, then once per month until off sirolimus, or more often if clinically indicated.

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

8. 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 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 of collaborating institution.

GVHD evaluation guidelines are as follows:

- History and physical exam (see **Appendix G**)
- Skin biopsy
- Schirmer's tear test
- Pulmonary function test
- Oral exam
- CXR
- Dietician assessment
- Gynecological departure assessment (adult female)

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

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

Table 11: Post-Transplant Evaluation

This is a recommended evaluation schedule.

See Tables 7-10 for pre-transplant evaluations. Additional lab instructions in Table 12.

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				

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 FHCRC patients only	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
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				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
MDS/ MPD	**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				

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 FHCRC patients only	**See comment*	**See comment*	**See comment*	**See comment*	**See comment*	**See comment*	**See comment*
	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 FHCRC patients only	**See comment*	**See comment*	**See comment*	**See comment*	**See comment*	**See comment*	**See comment*
	GVHD evaluation	Clinical	See text for details			x				

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				

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				

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				

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, if bone marrow not obtained	*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 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				

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	
	β-2 microglobulin	Clinical				x		x		
	LDH	Clinical			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				

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

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

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
	Imaging									
	Complete skeletal survey	Clinical						x		x
	Skeletal MRI	Clinical						x		x
	GVHD evaluation	Clinical	See text for details			x				

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 12: 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	Seattle Cancer Care Alliance (206) 288-7700
Pathology (*aspirate*)	Clinical	2mL bone marrow in EDTA formalin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Pathology (*biopsy*)	Clinical	1cm bone marrow in formalin **OR** mounted in paraffin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Flow Cytometry	Clinical	2mL bone marrow in green-top tube	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
Cytogenetics	Clinical	3mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
FISH	Clinical	2mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
PCR for bcr-abl and p190 and/or p210	Clinical	3mL bone marrow in lavender-top tube Label "protocol 2206"	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
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) 288-7060
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) 288-7060
Flow Cytometry	Clinical	10mL blood in green-top tube	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
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	SCCA Alliance Lab	Seattle Cancer Care Alliance (206) 288-2057
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	SCCA Alliance Lab	Seattle Cancer Care Alliance (206) 288-2057
PCR for bcr-abl and p190 and/or p210	Clinical	7mL blood in lavender-top tube Label "protocol 2206"	UW Molecular Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
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) 288-7060
ZAP – 70 by Flow cytometry (*pre-transplant only*)	Clinical	5mL blood in green-top tube	UW Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060

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

C. **Donor Evaluations**

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.

14. 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 P**).

B. **TBI**: TBI will be given in one 200-300 cGy fraction from linear accelerator at a rate of 6 - 7 cGy/min. 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.a.3 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.c.1. 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 R lists medications including voriconazole, posaconazole, and fluconazole 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 in Appendix E 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 P) 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 levels (fasting) should be drawn every two weeks through Day + 56, then monthly while on Sirolimus, or more often if clinically indicated. Cholesterol levels will be drawn at Day + 84 departure workup. In general, triglyceride levels remained below 1000 mg/dL. However, in 2/14 patients in our previous study, levels reached 2145 and 2152. 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.

E. **MMF**: See section 11.H.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.

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.b.4.

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 F and G**).
2. Recommended Treatment:
   a. Patients developing acute GVHD ≥ grade II off immunosuppression or while on a CSP taper:
      i. CSP 5 mg/kg PO q12hrs. If there is concern of GI absorption use IV route (1.5mg/kg q12hrs).
      ii. 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.
      iii. Patients may also be eligible for institutional trials of GVHD therapy.
   b. Patients who develop acute GVHD ≥ grade II prior to day +100:
      - Patients who develop acute GVHD ≥ grade II should receive prednisone (1-2 mg/kg/day) or intravenous equivalent. Continuation of sirolimus (Arms 2 and 3) beyond day 80 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. When steroids are tapered to less than 0.5 mg/kg, then a MMF taper should be initiated no sooner than day +100 and such that the completion of the taper is NOT prior to Day + 150. After successful discontinuation of MMF and corticosteroids, the suggested sequence for tapering CSP is to taper the CSP such that the completion of the taper is NOT prior to Day + 180 post transplant. After successful discontinuation of CSP, it is suggested that sirolimus should be tapered such that the completion of the taper is NOT prior to Day +365.

- 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, gancyclovir etc.), rejection, relapse or after DLI.

Patients with myelosuppression may be managed as follows:

1. Suspected MMF toxicity: refer to sections 11.H.b Guidelines for MMF dose adjustment above for management recommendations.
2. Suspected sirolimus toxicity: refer to sections 11.H.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 above for management recommendations.

15. Records

Clinical records will be maintained as confidentially as possible by all collaborating institutions. Collection of Case Report Forms (CRF) at standard intervals is the primary method of collecting data from collaborating centers. Clinical Statistics at FHCRC 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.

At the FHCRC, patient research files are kept in a locked room. They are maintained by the FHCRC data collection staff that is supervised by an A.R.T. Access is restricted to personnel authorized by the Division of Clinical Research.

16. Statistical Considerations

The primary objective of this study is to evaluate the efficacy of CSP, MMF and sirolimus in reducing the rate of grade II-IV acute GVHD in HLA class I or II mismatched nonmyeloablative transplants. The rate of grade II-IV acute GVHD in previous studies in this patient population has been approximately 70% at day 100. The rate of grade III-IV acute GVHD was approximately 25%.

Fifty-five patients will be enrolled. If grade II-IV acute GVHD occurs in 33 or fewer patients by day 100, then we can be at least 90% confident that the true rate of GVHD is less than 70%. The probability of achieving this outcome (power) is 95%, if the true rate of GVHD is lowered to 50%, and 81% if the true rate is 55%.

If grade III-IV acute GVHD occurs in 10 or fewer patients by day 100, then we can be at least 85% confident that the true rate of GVHD is less than 25%. The probability of achieving this outcome (power) is 81% if the true rate of GVHD is lowered to 15%.

Modified accrual target: As of November, 2015 the protocol is modified to increase target accrual to 80 patients, to enable separate evaluation of class I and class II mismatches. Although we have no reason to believe that the potential efficacy of the regimen will differ between classes, it will be of benefit if we can assert with some degree of confidence that acute GVHD is reduced for either kind of mismatch. Based on current accrual, class II mismatches comprise about 30% of enrollment; thus, with 80 patients we anticipate 55 patients with a class I mismatch and 25 patients with a class II mismatch. The statistical considerations for the group with class I mismatch will be as previously described above for the overall cohort. For the group with class II mismatch, 14 or fewer patients out of 25 with grade II-IV acute GVHD by day 100 will allow us to be at least 90% confident that the true rate is less than the 70% historical rate. The probability of achieving this outcome is 79%, if the true rate of acute GVHD is 50%. If grade III-IV acute GVHD by day 100 occurs in 4 or fewer patients, then we can be at least 80% confident that the true rate is less than the historical rate of 25%. The probability of achieving this outcome is 68% if the true rate is lowered to 15%. Stopping rules will continue to be monitored for the overall patient group, regardless of type of mismatch.

Although we have no expectation that the addition of sirolimus to the immunosuppressive regimen will impact graft rejection, a stopping rule for graft rejection will be imposed, with a threshold rate of 5% by day 100. 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. The study will stop whenever there is reasonable evidence that the true rate of graft rejection exceeds this threshold. Reasonable evidence will be taken to mean that the lower bound of a 1-sided 90% confidence interval for the rate of graft rejection is greater than 5%. Operationally, this rule will be evaluated at least every 5 patients, and will be triggered by the following outcomes: 2 rejections within the first 10 patients, 3 rejections within the first 20 patients, 4 rejections within the first 35 patients, 5 rejections within the first 45 patients, 6 rejections within the first 60 patients, 7 rejections within the first 75 patients, or 8 rejections in any number of patients.

A stopping rule will also be imposed for non-relapse mortality (NRM) at day 100, which was approximately 20% in protocol 1591. The study will suspend accrual for review by the DSMB whenever there is reasonable evidence that the true rate of NRM exceeds the historical rate. Reasonable evidence will be taken to mean that the lower bound of a 1-sided 90% confidence interval for the rate of day 100 NRM is greater than 20%. Operationally, this rule will be evaluated at least every 5 patients, and will be triggered by the following outcomes: 3 NRM deaths within the first 5 patients, 5 deaths in 10 patients, 6 deaths in 15 patients, 7 deaths in 20 patients, 9 deaths in 25 patients, 10 deaths in 30 patients, 11 deaths in 35 patients, 12 deaths in 40 patients, 13 deaths in 45 patients, 15 deaths in 50 patients, 16 deaths in 55 patients, 17 deaths in 60 patients, 18 deaths in 65 patients, 19 deaths in 70 patients, 20 deaths in 75 patients, or 22 deaths in 80 patients.

Stopping rules will be imposed for:

- Graft Rejection of 5% by day 100
- NRM of > 20% at day 100

Enrollment may continue pending evaluation of these outcomes in currently enrolled patients, but the outcome of these additional patients will not override the stopping rule if triggered in an earlier number. The operating characteristics of the stopping rules are provided in the table below.

True rate of graft rejection	Probability of stopping[1]	Average n enrolled[1]	True rate of Day 100 NRM	Probability of stopping[1]	Average n enrolled[1]
0.05	21%	69	.20	25%	67
0.10	75%	42	.25	58%	52
0.15	97%	23	.30	86%	35
0.20	>99%	15	.35	98%	24

[1] estimated from 10,000 Monte Carlo simulations

17. Data and safety Monitoring Plan

FHCRC Protocol 2206 Data and Safety Monitoring Plan

1. Monitoring the progress of trials and the safety of participants
Protocol 2206 is a multi-institutional clinical trial that is monitored by the principal investigator (PI), Dr. Sandmaier, 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 mismatched donor HCT and the updated data are presented at Mixed Chimerism Meetings (includes co- investigators).

Please see **Appendix I** 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 trial coordinators at collaborating centers or the local PIs will fax an official report of a SAE to the coordinating center (FHCRC) within ten days. The SAE report is reviewed by Dr. Sandmaier. If the SAE meets the FHCRC criteria for expedited reporting then an official signed report is submitted to the FHCRC Institutional Review Office (IRO). All deaths, regardless of the cause, are reported to the IRB. Protocol 2206 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 (FHCRC) along with those officially reported to the FHCRC 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 FHCRC 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 FHCRC 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 FHCRC, 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.

Protocol 2206 will be a multi-institutional protocol and all collaborating centers sign an agreement with the FHCRC stating that data generated from patients from the protocol will be reported accurately in a timely manner to the FHCRC. All centers have an IRB that reviews the protocol and that the local PIs contact when an adverse event on the protocol occurs. Most of the centers have internal auditing mechanisms that assure accurate assessment of clinical outcomes. Clinical outcome data are summarized and transmitted from collaborating centers as CRFs. 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 from the collaborating centers at defined time points (100 days, 6 months, and yearly). The local PI reviews the official CRF and primary source documents. When the CRFs are verified, they are signed and the data are entered into a central database managed by the FHCRC trial coordinator.

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

The adverse event reporting in this multi institution clinical trial will follow the FHCRC Guidelines for s SAE reporting. These guidelines (attached in **Appendix I**.) detail the expedited reporting requirements, definitions of particular events. All SAEs that meet expedited reporting 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. For patients being cared for at the FHCRC, 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 FHCRC 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 toxicity. Furthermore, an additional safeguard for adverse event analysis and reporting in this protocol is provided by stopping rules that are monitored at least every 10 patients in each arm. All collaborating PIs have fulfilled all NIH requirements for training in human subjects protection.

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

Collaborating sites send signed consents, eligibility forms, and CRFs with source documents demonstrating eligibility, treatment, and serious adverse events (if applicable) to the study staff. These are reviewed for eligibility, adherence to the protocol, accuracy, and completeness by the study staff. Queries are sent to the collaborating investigators if CRFs are inaccurate or incomplete.

The study is monitored under the FHCRC Monitoring Plan. The FHCRC Data and Safety Monitoring Plan details the full scope and extent of monitoring and provides for immediate action in the event of the discovery of major deviations.

18. Targeted/Planned Enrollment

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex / Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	1	2	3
Not Hispanic or Latino	33	44	77
Ethnic Category Total of All Subjects*	34	46	80
Racial Categories			
American Indian / Alaska Native	0	0	0
Asian	1	2	3
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	1	2
White	32	42	75
Racial Categories: Total of All Subjects*	34	46	80

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

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APPENDICES – TABLE OF CONTENTS

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
THE LANSKY PLAY-PERFORMANCE SCALE
(FOR USE WITH PERSONS AGES 1 – 16 YEARS)

SCORE (%)	DESCRIPTION
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both, greater restrictions of, and less time spend in play activities
60	Up and around, but minimal active play, keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play; able to participate in all quiet play activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	Unresponsive
0	Dead

Appendix D

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 PBSC products will only be 2-5% of the product volume before infusion, the small quantity may cause a hemolytic transfusion reaction. According to the FHCRC 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 PBSC 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 PBSC products within 2 hours of depletion.
Expected Results: Red blood cell depleted PBSC 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 PBSC inoculum, recipients may be at much greater risk for a delayed type of hemolysis that can be severe. PBSC products contain < 200ml of plasma according to FHCRC policy and no deleterious effects have been observed so far. However, if donors show an anti-recipient titer ≥ 1:256, the PBSC 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 PBSC 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 PBSC 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 E

Infectious Disease Guidelines: Monitoring, Prevention and Treatment

Herpes Simplex and Varicella Zoster Virus Prevention and Treatment

hsv-vzv.pdf

CMV Prevention: Surveillance and Preemptive Therapy

cmvprevention.pdf

CMV Disease: Diagnosis and Treatment

cmvdiseasetreatmen
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Antifungal Therapy Guidelines

antifungal_therapy.p
df

Pneumonia / Pneumocystis Carinii Prophylaxis

pneumocystisjiroveci
.pdf

Antibiotic Prophylaxis for Encapsulated Bacteria in Allogeneic Patients with Chronic GvHD Requiring Immunosuppressive Therapy

antibioticprophylaxisf
orencapsulatedbacte

Vaccinations

Vaccines

Foscarnet

foscarnet.pdf

APPENDIX F

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 G

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. Classification of Chronic GVHD

The purpose of this classification is to identify patients with cGVHD who need long-term systemic immunosuppression according to clinical and laboratory findings and risk factors at the time of initial diagnosis. In addition, a morbidity scale has been developed to help grade the severity of manifestation of chronic GVHD (Appendix D) at the time of diagnosis, when changes in treatment are made and when assessing treatment response.

1. **Chronic GVHD not requiring systemic treatment: mild abnormalities involving a single site, with platelet count >100,000 and no steroid treatment at the onset of chronic GVHD**
   a) Oral abnormalities consistent with cGVHD, a positive skin or lip biopsy, and no other manifestations of cGVHD
   b) Mild liver test abnormalities (alkaline phosphatase ≤2 x upper limit of normal, AST or ALT ≤3 x upper limit of normal and total bilirubin ≤1.6) with positive skin or lip biopsy, and no other manifestations of cGVHD
   c) Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of body surface area (BSA), dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy, and no other manifestations of cGVHD
   d) Ocular sicca (Schirmer's test ≤5mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of cGVHD
   e) Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of cGVHD

2. **Chronic GVHD requiring systemic treatment: more severe abnormalities or involvement of multiple sites, or platelet count <100,000, or steroid treatment at the onset of chronic GVHD**
   a) Involvement of two or more organs with symptoms or signs of cGVHD, with biopsy documentation of cGVHD in any organ
   b) ≥15% base line body weight loss not due to other causes, with biopsy documentation of cGVHD in any organ
   c) Skin involvement more extensive than defined for clinical limited cGVHD, confirmed by biopsy
   d) Scleroderma or morphea
   e) Onycholysis or onychodystrophy thought to represent cGVHD, with documentation of

cGVHD in any organ
f) Decreased range of motion in wrist or ankle extension due to fasciitis caused by cGVHD
g) Contractures thought to represent cGVHD
h) Oral involvement with functional impairment, refractory to topical treatment
i) Vaginal involvement with functional impairment, refractory to topical treatment
j) Bronchiolitis obliterans not due to other causes
k) Positive liver biopsy; or abnormal liver function tests not due to other causes with alkaline phosphatase >2 x upper limit of normal, AST or ALT >3 x upper limit of normal, or total bilirubin >1.6, and documentation of cGVHD in any organ
l) Positive upper or lower GI biopsy
m) Fasciitis or serositis thought to represent cGVHD and not due to other causes

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 (ichtyosis), 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.

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 Research Center Standard Practice Manual, Section X, Chronic Graft Versus Host Disease (GVHD), Nov/2003 Version

Appendix H
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.

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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 I

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 FHCRC 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 FHCRC. 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 FHCRC Principal Investigator. The FHCRC 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. Registrations

Collaborating Institutions: The principal investigator of the collaborating institution who will register the patient with the FHCRC will identify eligible patients. Registration will include completion of the eligibility checklist/demographic form. This form will be faxed to (206-667-5378) prior to treatment initiation. Patients should be registered prior to treatment initiation for valid registration

IV. 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 Research Center.

APPENDIX I cont'd.

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

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.

FHCRC is acting as the Coordinating Center for this multi-institutional study, and it is the responsibility of the FHCRC Principal Investigator (or designee) to complete the FHCRC Serious Adverse Event Report for all serious adverse events that meet the expedited reporting requirements that are received from the participating sites. It is the responsibility of the FHCRC Principal Investigator to notify the sponsor, NIH, FDA or other agencies of serious adverse events as required in the protocol.

Procedure for Reporting Serious and Unexpected Adverse Events (SAE) from Participating Sites
Regulations defining the responsibilities for reporting serious and unexpected adverse reactions are defined above. SAEs or any death regardless of cause (serious, unexpected, and related/possibly related) within 180 days after HCT must be reported to the FHCRC Investigator within 10 days of learning of the event. The immediate telephone report must be followed by faxed comments to the FHCRC trial coordinator at **(206) 667-5378.** This will be followed by detailed written report (See Appendix "J") within 10 days. The report must include the date and time of onset, severity and duration of the event, the relationship to the study, the treatment given and eventual outcome. Follow-up information to a SAE report must be submitted as soon as the relevant information is available.

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 start of conditioning to day100 during the study will be recorded on the Case Report Form (**Appendix M**). These adverse events which are observed by the Investigator or reported by the patient, whether or not attributed to the study, will be recorded on the Case Report Form using the selected (for this protocol) NCI Common Toxicity Criteria (NCI-CTC) version 4 (**Appendix P**) . 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 FHCRC Investigator within 10 days of learning of the event as described above for reporting of SAE.

APPENDIX I cont'd.

V. Case Report Forms

Clinical outcome data are summarized and transmitted from collaborating centers as CRFs. Case report forms must be completed for all patients registered onto the protocols and submitted to the FHCRC 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 from the collaborating centers at defined time points (day 28, 56, 84, 100, 6 months, 1 year, 18 months and annually). The local PI reviews the official CRF and primary source documents. For Outside Centers, case report forms are expected to be submitted no later than 30 days following the scheduled follow up date. When the CRFs are verified, the data is entered into a central database managed by the trial coordinator.

VI. Protocol Monitoring

As the coordinating center, FHCRC 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 FHCRC 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 J

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

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ ☐ FHCRC/SCCA ☐ Other

Gender: ☐ Male ☐ Female Age: _____

FHCRC 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 _____ #2 _____

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

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 SCCA outpatient clinic, a copy of this Protocol Modification Form and any supporting documents to be reviewed and approved, will be forwarded to the FHCRC's Institutional Biosafety Committee (IBC) by the Protocol Office (Mailstop: LM-230).

_____ _____
Signature of Principal Investigator Date:

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

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ Date of Report: _____

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

APPENDIX K

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 L

Protocol 2206 Patient Demographics and Eligibility Form

Please Fax this completed form to (206) 667-5378 for patient registration.
Questions regarding eligibility should go to Brenda Sandmaier, M.D., 206-667-4961.

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)

Status at Transplant:_____

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.

☐ TBI 2 Gy

OR

☐ TBI 3 Gy:

PI Signature: _____ Date: _____

Protocol 2206 Eligibility
I) Inclusion Criteria:

1) Yes ☐ No ☐ Patient signed and dated consent form.
Date: _____
Date of IRB approval of consent form: _____
IRB file: _____

2) Yes ☐ No ☐ Related or unrelated donors who are prospectively:

a. Mismatched at antigen level for any single class I locus (HLA-A, -B or –C) ± an additional class mismatch at the allele level

OR

mismatched at the allele level for any 2 class I loci (if typed at the molecular level)

OR

mismatched at the antigen or allele level for class II loci HLA-DRB1 and/or –DQB1. Must be matched for at least one DRB1 allele and one DQB1 allele

b. There is a likelihood of rapid disease progression while HLA typing and results of a preliminary search and the donor pool suggests that a 10/10 HLA-A,B,C, DRB1 and DQB1 matched donor will not be found

c. There is no HLA-A, -B, or –C one locus allelic mismatched donor available

d. If the patient is homozygous at the mismatched HLA class I 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

e. There is no mismatch both at a class I and II locus

f. There is no indication for an autologous transplantation as a treatment option

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

Patient					
A: _____	A: _____	C: _____	C: _____	B: _____	B: _____
DRB1: _____	DRB1: _____	DQB1: _____	DQB1: _____		
Donor					
A: _____	A:_____	C: _____	C: _____	B: _____	B: _____
DRB1: _____	DRB1: _____	DQB1: _____	DQB1: _____		

One of the following criteria questions (3-6) must be marked "Yes" for the patient to enter on 2206

3) Yes ☐ No ☐ Ages >50 years with hematologic malignancies treatable by related or unrelated allogeneic HCT.

4) Yes ☐ No ☐ Ages ≤ 50 years of age 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 (>40% risk of TRM). This criterion can include patients with a HCT-CI score of ≥1 (**see Appendix Q**). Transplants should be approved for these inclusion criteria by the principal investigators at the collaborating centers and at FHCRC.

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

*****. *All children < 12 years must be discussed with the FHCRC PI (Brenda Sandmaier, MD 206-667-4961) prior to registration.*

5) Yes ☐ No ☐ Ages ≤ 50 years of age with chronic lymphocytic leukemia (CLL.

6) Yes ☐ No ☐ Ages ≤ 50 years of age with hematologic diseases treatable by allogeneic HCT who refuse a high dose HCT. Transplants must be approved for these inclusion criteria by the principal investigators at the collaborating centers and at FHCRC.

One of the following criteria questions (7-17) must be marked "Yes" for the patient to enter on 2206. The following diseases will be permitted although other diagnoses can be considered if approved by PCC or the participating institution's patient review committees and the principal investigator.

7) 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 allogeneic HCT, or after failed autologous HCT.

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

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

10) 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; 4) Patients with a diagnosis of CLL (or small lymphocytic lymphoma) that progresses to prolymphocytic leukemia (PLL); or 5) T-cell CLL or PLL.
Describe which inclusion is specific for this patient:_____.

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

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

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

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

15) 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.

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

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

II) EXCLUSION CRITERIA:

Each of the following questions must be marked "No" Or "NA" for the patient to enroll on 2206.

18) Yes ☐ No ☐ Patients for whom the best available donor is mismatched at both HLA class I and class II

19) Yes ☐ No ☐ A positive cross-match exists between the donor and recipient.

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

21) Yes ☐ No ☐ NA ☐ Patients with a diagnosis of CMML.

22) Yes ☐ No ☐ NA ☐ Patients with RAEB-2 who have not received myelosuppressive chemotherapy i.e. induction chemotherapy.

23) Yes ☐ No ☐ CNS involvement with disease refractory to intrathecal chemotherapy. For LP requirement, see Appendix N.

24) Yes ☐ No ☐ NA ☐ Presence of circulating leukemic blasts (in the peripheral blood) detected by standard pathology for patients with AML, ALL or CML.

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

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

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

28) 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.

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

All patients receiving antifungal therapy voriconazole, posaconazole, or fluconazole must have sirolimus dosing reduced according to the Standard Practice Antifungal Therapy Guidelines in Appendix E.

PI Signature: _____ Date: _____

30) Yes ☐ No ☐ Cytotoxic agents for "cytoreduction" with the exception of imatinib (imatinib mesylate), cytokine therapy, hydroxyurea, low dose cytarabine, chlorambucil, or rituxan within three weeks of the initiation of conditioning

31) 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%, TLC <40%, FEV1 <40% and/or receiving supplementary continuous oxygen.
NOTE: The FHCRC PI of the study must approve of enrollment of all patients with pulmonary nodules. If unable to perform complete PFTs, patients will be excluded if their oxygen saturation is <95% with a formal six-minute walk test (ambulatory oximetry).

PI Signature: _____ **Date**: _____

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.

32) Yes ☐ No ☐ Karnofsky score < 60 or Lansky score <50.

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

34) Yes ☐ No ☐ HIV positive patients.

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

Note – the HCT-Comorbidity score is: _____

☐ FHCRC Patients:

Signature of person completing form: _____ Date: _____

Signature of Principal Investigator: _____ Date: _____

OR

☐ Outside Center Patients:

Signature of person completing form: _____ Date: _____

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

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

APPENDIX M
Core Case Report Forms (CRFs)

Acrobat Document

APPENDIX N

Intrathecal Diagnostics and Therapeutics

intrathecaltherapy-combined.pdf

Appendix O
HLA MATCHING REQUIREMENTS FOR DONORS AT THE SCCA / FRED HUTCHINSON ALLIED SYSTEM

Human Leukocyte Antigen (HLA) Terminology. The HLA region consists of genes that encode two classes of HLA molecules. **HLA class I molecules**, HLA-A, -B, and –C, are composed of a single glycoprotein chain that is expressed in association with ®2-microglobulin on most tissue cells. **HLA class II molecules**, HLA-DR, -DQ, and –DP, are heterodimers consisting of ⟨ and ® glycoprotein chains. HLA class I and HLA class II molecules are highly polymorphic.

HLA Typing Methods. At the Seattle Cancer Care Alliance Clinical Immunogenetics Laboratory (CIL) DNA-based methods of HLA-A, B, C, DRB1, DQB1 typing are now performed routinely. **High resolution** typing is required to define individual alleles and the level of mismatching between donor and recipient. **High resolution** data are reported with four or more digits (e.g., A*0201, A*0205, B*1504, or DRB1*0401**). A current listing of recognized HLA alleles and their sequences can be found at the Immunogenetics/HLA sequence database website at** www.anthonynolan.org.uk/HIG/data.html**.**

Initial typing reports obtained through the international marrow donor registries may consist of **intermediate resolution** typing. **Intermediate resolution** defines alleles in groups of related families historically defined as ***antigens*** by alloantisera. **Intermediate resolution** typing results are reported as two digits (e.g., A*02, B*15, or DRB1*04). In cases where the HLA-A, B and C loci are typed at intermediate resolution and high resolution data are not available, it should be understood that unidentified allele disparity might be present.

Donor Selection. Final selection of donor should be based upon results of **high resolution** typing of HLA-A, B, C, DRB1, DQB1 alleles. Cross match assay is not required when high resolution typing indicates matching for HLA-A, B, C, DRB1 and DQB1 AND the platelet reactive antibody (PRA) screen is not elevated (defined as ≤10%). A negative cross match test result is required for final donor selection in the following situations: 1) PRA screen is positive (>10%), or 2) high resolution typing indicates mismatching for one or more HLA-A, B, C, DRB1 and DQB1 alleles. A positive anti-donor cytotoxic crossmatch absolutely excludes the donor.

Donor Selection Criteria. Protocols and treatment plans must specify donor inclusion and exclusion criteria, using terminology indicated below.

Donor inclusion criteria **must specify** 1) the allowable genetic relationship between the patient and donor (related and/or unrelated), 2) the allowable limits of mismatch, and if applicable 3) any modification of mismatch criteria according to type of disease or patient characteristics.

Acceptable levels of recipient-donor mismatch for research related treatment protocols or standard treatment plans include the following:

Allele-match for HLA-A, B, C, DRB1 and DQB1.
Single allele disparity for HLA-A, B, C, or DRB1 or DQB1
Two allele disparities for HLA-A, B, or C.
Single allele disparity for HLA-DRB1, with or without a single DQB1 antigen or allele disparity.
Single antigen plus single allele disparity for HLA-A, B, or C.

Appendix O (cont'd)

HLA MATCHING REQUIREMENTS FOR DONORS AT THE SCCA / FRED HUTCHINSON ALLIED SYSTEM

hla_testing_donors-recipients.pdf

APPENDIX P

Adapted from
COMMON TOXICITY CRITERIA (CTC)
Version 4.0

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 renal failure)	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 Q

The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI) **9/7/10**

Assign scores appropriately if the patient has any of these comorbidities

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 R

CLINICALLY SIGNIFICANT INDUCERS/INHIBITORS OF CYTOCHROME P450 ENZYME SYSTEM

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

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

APPENDIX S

Standard Donor Consent

standard donor consent.pdf

Appendix T

Weight / Adjusted Body Weight for Drug Dosing

weight_for_drug_dosing.pdf

Appendix U

COORDINATING CENTER FUNCTIONS

Outside Center – PI Communication in Hematologic Malignancies

I. Study Management, data analysis, and Data and Safety Monitoring

a. Study Management:

i. Each local PI is responsible for selection, training and oversight of local study coordinators

ii. The Coordinating Center registers subjects on the study and assigns study IDs

iii. One copy of the research data is retained by the site. Another data set (identified only by study IDs) is transmitted to the Coordinating Center to create the master data file. All data are kept in locked areas and password protected databases accessible only to study staff

iv. The quality of data is monitored in an ongoing fashion with the study team and corrective action plans instituted as necessary

b. Data Analysis:

i. Study staff review data for completeness as it is submitted by the sites

ii. The study statistician is responsible for data cleaning and the conduct of analyses as outlined in the protocol and grant

c. Data Safety and Monitoring:

i. The trial coordinators at collaborating centers or the local PIs will fax an official report of an SAE (as defined by the protocol) to the Coordinating Center within ten days.

ii. The SAE report is reviewed by the Overall PI. If the SAE meets the FHCRC criteria for reporting then an official signed report is submitted to the IRB

iii. An independent DSMB will meet at six-month intervals and all outcome data is reviewed including all adverse events and SAEs reported to the Coordinating Center along with those officially reported to the IRB

iv. A report from the DSMB is submitted to the IRB as well as the trial coordinators/local PIs participating in the protocol

II. Protocol and informed consent document management

a. A master protocol is maintained by the Coordinating Center and distributed to the sites for customization and local IRB review

b. All protocol and consent modifications initiated by the Coordinating Center are sent to the Collaborating Sites following approval by the Coordinating Center IRB, for review and approval by the local IRB

c. Changes required by local IRBs are reviewed by the Coordinating Center and approved prior to implementation at local sites

III. Assurance of local IRB OHRP-approved assurance

a. Each site provides their OHRP assurance number and evidence of IRB certification

b. Study staff monitor maintenance of institutional assurance and IRB certification

IV. Assurance of local IRB approvals

a. The Coordinating Center maintains copies of the most current collaborating site Consent Forms and IRB approval documentation
b. No site may enroll subjects until the Coordinating Center has received confirmation of local IRB approval
c. Each site is responsible for preparation and submission of their continuing reviews. Any changes to the protocol or consent form will be communicated to the Coordinating Center
d. Sites are required to have active IRB approvals to participate in any study related activities

V. Any substantive modification by the Collaborating Institution related to risks or alternative procedures is appropriately justified

a. The Coordinating Center reviews any modifications to consent forms to ensure that site consents do not delete or change the basic or additional elements or alternatives required in the sample consent form

VI. Informed consent is obtained from each subject in compliance with HHS regulations

a. Subjects must provide written informed consent prior to study participation
b. The Coordinating Center verifies eligibility and signed consent prior to assigning a study ID number

Appendix V

Radiotherapy Treatment Guidelines per Standard Practice

TBI_Adult_Non_Myeloablative.pdf

TBI_Pediatric_NON_Myeloablative.pdf