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1. A Randomized Phase III Study to Determine the Most Promising Postgrafting Immunosuppression for Prevention of Acute GVHD after Unrelated Donor Hematopoietic Cell Transplantation using Nonmyeloablative Conditioning for Patients with Hematologic Malignancies: A Multi-Center Trial

Investigators:

Investigators		**Phone**
Brenda M. Sandmaier, MD	Member, FHCRC, Professor of Medicine, UW	(206) 667-4961
David Maloney, MD, PhD	Member, FHCRC, Professor of Medicine, UW	(206) 667-5616
Mary E. D, Flowers, MD	Member, FHCRC, Professor of Medicine, UW	(206) 667-5115
Effie Petersdorf, MD	Member, FHCRC, Professor of Medicine, UW	(206) 667-5244
Ann Woolfrey, MD	Member, FHCRC, Professor of Pediatrics, UW	(206) 667-4453
Paul J. Martin, MD	Member, FHCRC, Professor of Medicine, UW	(206) 667-4798
Marco Mielcarek, MD	Associate Member, FHCRC, Associate Professor, UW	(206) 667-2827
Rainer Storb, MD	Member, FHCRC, Professor of Medicine, UW	(206) 667-4407
Statistician:		
Barry Storer, PhD	Member, FHCRC, Affiliated Associate Professor of Biostatistics, UW	(206) 667-6151
Radiation Oncologist:		
Ralph Ermoian, MD	Assistant Professor, Radiation Oncology, UW	(206) 589-4100

Research Nurses: Michelle Bouvier, RN (206) 667-6993, pager (206) 995-7658

Additional Performance Sites	**Investigators**	**Emergency Phone Numbers**
University of Colorado	Jonathan Gutman, MD	(303) 266-0763
University of Utah	Michael Boyer, MD	(801) 585-3229
Rigshospitalet	Søren Lykke Petersen, MD	+45 35 45 1134
Aarhus Hospital	Gitte Olesen, MD	+45 78 46 1152
Emory University	Amy Langston, MD	(404) 778-4189
HealthONE Presbyterian St. Luke's Medical Center	Michael Maris, MD	(720) 754-4800
Veterans Affairs Puget Sound Health Care System	Thomas Chauncey, MD, PhD	(206) 762-1010
University of Tubingen	Wolfgang Bethge, MD	49-7071-2982711
University of Leipzig	Georg-Nikolaus Franke, MD	0341-97-13842
University of Koln	Kai Huebel, MD	0221-4785133

Emergency Phone FHCRC: (206) 598-8902, FAX (206) 598-4034
SEATTLE CHILDREN'S: (206) 987-2000*, FAX (206) 987-2764
*Ask Seattle Children's Operator for "Transplant Provider On-Call"

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2. Introduction

Hematopoietic cell transplantation (HCT) from unrelated donors after nonmyeloablative conditioning is effective therapy for patients with advanced hematologic malignancies who are considered at high risk for treatment related mortality with high dose conditioning because of older age or comorbidities [1,2]. The nonmyeloablative regimen currently used consists of fludarabine (FLU) (90mg/m^2), 2 Gy total body irradiation (TBI) and post-grafting immunosuppression with a calcineurin inhibitor [cyclosporine (CSP) or tacrolimus] and mycophenolate mofetil (MMF). For patients transplanted with HLA-matched unrelated donors CSP is started on day –3 on MMF on day 0. This immunosuppressive regimen has been proven successful in enabling sustained engraftment of granulocyte colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC) from unrelated donors, with a rejection rate as low as 5 % [3]. However, graft versus host disease (GVHD) rates remain high with cumulative incidences of grade II-IV acute and extensive chronic GVHD of 52% and 40%, respectively. In a analysis of GVT effects in 322 patients with hematological malignancies, multivariate analyses showed an association between extensive chronic GVHD and a decreased risk of relapse/progression (p=0.003), while acute GVHD only was associated with non-relapse mortality without any statistically significant impact on the risk of relapse/progression [4]. 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. Protocol 1938 was a randomized phase II study designed to determine the most promising postgrafting immunosuppression for prevention of acute GVHD after unrelated donor transplantation using nonmyeloablative conditioning. Patients were randomized into three arms, where two of the arms consisted of different MMF and tacrolimus schedules, while the third arm had sirolimus added to the combination of tacrolimus and MMF. By May 2010 183 patients had been accrued. The cumulative incidence of grade II-IV acute GVHD was significantly lower (p=0.03) in the sirolimus arm (45%) as compared to the reference arm (65%), which consisted of tacrolimus and MMF, administered in a schedule similar to the above mentioned current standard of care. In the analysis of the impact of sirolimus on the individual grades of acute GVHD, the benefit was only observed for grade II. No difference between treatment arms was observed for grade III-IV acute GVHD, chronic GVHD, nonrelapse mortality and relapse/progression. The rationale for substituting tacrolimus for CSP in protocol 1938 was derived from two large randomized trials of myeloablative conditioned transplants showing decreased incidences of acute and chronic GVHD in the favor of tacrolimus as compared to CSP, both in combination with methotrexate (MTX)[5,6]. However, when the results of protocol 1938 were compared to a historical cohort 174 unrelated non-myeloablative transplants treated with a combination of CSP and MMF, no difference was observed in any of the outcome parameters. Thus, suggesting that the substitution of CSP for tacrolimus did not entail additional GVHD control. In a retrospective analysis performed in recipients of high-dose conditioning, there was a finding that there was a higher risk of grades 3-4 GVHD in the tacrolimus cohort as compared to the CSP cohort. However, this analysis was confounded by the fact that the two cohorts differed both in time (majority of patients before 2004 were treated with CSP and with tacrolimus after 2004). Also, starting in 2003, all patients were treated with ursodiol which significantly reduces the rate of grades 2-4 and 3-4 acute and chronic GVHD (personal communication, Paul Martin).

The results using triple therapy with the addition of sirolimus to the “standard” immunosuppressive regimen was very promising and there was a significant reduction in acute GVHD. The goal of the current protocol is to compare in a phase III randomized trial, the effectiveness of 2 GVHD prophylaxis regimens in preventing acute grades II-IV GVHD. Both arms will use CSP until day 96 then tapered off by day 150. Arm 1 (similar to the former unrelated donor nonmyeloablative protocol 1938 Arm 2) will be the “reference arm” in which MMF is given tid until day 30, then bid until day 150 with a taper through day 180. In Arm 2 MMF will be given through day 40, and then discontinued without a taper and sirolimus will be given until day 150 with a taper though day 180. The current protocol’s primary objective is to reduce the incidence of acute GVHD after unrelated nonmyeloablative HCT.

3. Background

The proposed approach to establish sustained engraftment and GVHD control after nonmyeloablative conditioning in this protocol is derived from preclinical work performed at the FHCRC in the canine model[7-9] and clinical protocols 1463, 1641, 1668 and 1938.

A. Postgrafting immunosuppression in the canine model with CSP and MMF

The canine studies demonstrated that the use of immunosuppression after transplant with CSP and MMF could substitute for pre-transplant TBI otherwise required as pre-transplant immunosuppression for establishing allografts in major histocompatibility complex (MHC)-identical littermate recipients. In these studies, the administration of CSP for 35 days post-transplant resulted in stable engraftment of marrow from 7 of 7 MHC-identical littermates after conditioning with 450 cGy of TBI [10]. In contrast, without post-grafting CSP, 60% of dogs rejected their grafts after this radiation dose. In subsequent studies using marrow grafts from MHC-identical littermates given sublethal TBI (200 cGy), CSP alone was ineffective for establishing donor grafts [9]. However, combination therapy consisting of MMF or methotrexate (MTX) and CSP was successful in achieving stable grafts. The combination of MMF/CSP proved more effective than MTX/CSP. Of the 12 dogs that received MMF/CSP, 11 became long-term stable mixed chimeras without GVHD. Given that comparable rate of engraftment in dogs not administered post-grafting immunosuppression could only be achieved with 920 cGy, the combination of CSP/MMF given post-grafting appeared to provide immunosuppression approximately equivalent to that achieved with 720 cGy pre-transplant TBI[10].

B. Clinical FHCRC protocols

All patients enrolled in protocols 1463, 1641, 1668 and 1938 were transplanted with unrelated donors after nonmyeloablative conditioning with fludarabine 90mg/m^2 and 2 Gy TBI. Three patients with CML on protocol 1668 received 3 Gy of TBI.

i. Protocol 1463[2] and 1641[3]

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). Postgrafting 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 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 half-life 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% when MMF was given twice a day (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. For MDS/MPS patients the 1-year non-relapse and relapse related mortalities were 25% and 35%, respectively, 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.

ii. FHCRC Protocol 1668[11]

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 [12,13]. Conversely, antimetabolites such as MMF could delete autoreactive T cells by inducing apoptosis [14,15]. 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 progression free 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 protocol 1668 (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 protocol 1668 patients versus 52% and 15% among historical patients, respectively. Among the protocol 1668 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[11].

iii. FHCRC Protocol 1938[11]

The goal of Protocol 1938 was to evaluate in a phase II randomized trial, which of three immunosuppression drug combinations was most promising in preventing acute GVHD. **Figure 1** shows the treatment schema. Each of the combinations used MMF every 8 hours through day 30 and then MMF was given every 12 hours. The duration of MMF administration varied between the three arms. In each arm CSP was substituted for tacrolimus based on the previously observed lower incidence of acute GVHD when MTX was combined with tacrolimus instead of CSP after high-dose allogeneic HCT[16]. .Also patients given tacrolimus in those randomized studies who developed chronic GVHD required significantly shorter periods of therapy before chronic GVHD resolved in an analysis carried out in the FHCRC only patients. However, this finding did not hold out in subsequent analysis including the patients from the other Centers that participated in the trial (personal communication, Paul Martin). Arm 1 (similar to protocols 1463 and 1641) was the “reference arm” that gave MMF until day 40 with a taper over 2 months and tacrolimus until day 100 with a taper over 80 days. Arm II and III discontinued tacrolimus on day 100 with a taper over 50 days and on day 150 MMF was tapered off over a month. In Arm III, sirolimus was added to the GVHD prophylaxis regimen until day 80 in an attempt to further prevent acute GVHD. By May 2010 183 patients

had been accrued (arm #1 (reference), n=60; arm #2, n=61: arm #3, n=61), which were transplanted with PBSC from unrelated donors (10/10 HLA match in 91% and single HLA class I mismatch in 9%) after nonmyeloablative 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 1). 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 1A). Detailed analysis showed that the benefit of adding sirolimus restricted to lowering the incidence of grade II acute GVHD (figure 1B). 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 1C-D). When the results of protocol 1938 were compared to a historical cohort 174 unrelated nonmyeloablative transplants treated with a combination of CSP and MMF, no difference was observed in the cumulative incidences of nonrelapse mortality, relapse/progression, and acute (figure 1A) 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.

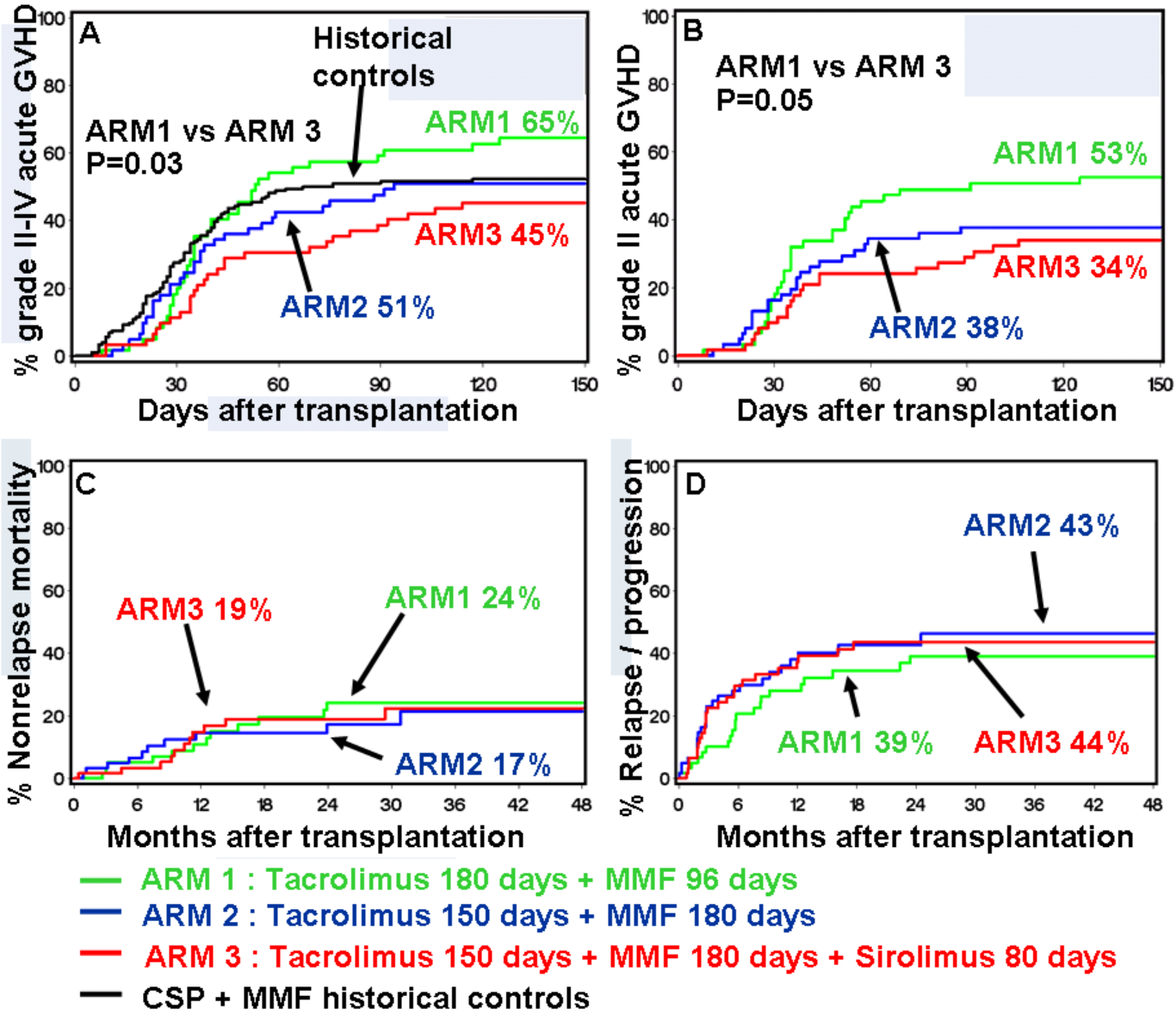

Figure 1: Results from Interim Analysis of Protocol 1938

C. 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)[4]. 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.

D. Sirolimus (Rapamune ®)

1. Mechanism of action.

i. Immunomodulatory effect. Sirolimus was isolated in a discovery program for novel antifungal agents. It is a macrocyclic lactone fermentation product of Streptomyces hygroscopicus, an actinomycete that was isolated from a soil sample collected from Rapa Nui (Easter Island). Although, the activity of sirolimus depends on its binding to the same class of cytosolic binding proteins (immunophilins) as CSP and tacrolimus, its mechanism of action is unique. The complex of CSP or tacrolimus with their respective immunophilins inhibit calcineurin, which in turn impairs signaling through the T-cell receptor, reducing the expression of cytokines important for the antigen specific expansion of T-cells (e.g. IL-2, IL-3, IL4 and TNFα.), hereby arresting their cell cycle in G_0 to G_1. Sirolimus has no effect on the calcineurin pathway, but inhibits the mammalian target of rapamycin (mTOR) protein kinase, which promotes cell proliferation and is a key regulatory kinase in cell cycle control. In contrast to CSP and tacrolimus inhibition of T-cell receptor induced activation and cytokine secretion, the sirolimus-immunophilin complex inhibits the T-cell's response to cytokines, hereby arresting the cell cycle at a later stage (G_1 to S phase) [17]. 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 $CD4^+CD25^{high}$ [18,19]. 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[20,21]. The preferential expansion of T_{reg} is attenuated when sirolimus is used in combination with CSP[19]. 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 [22-24].

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[25].

ii. Viral amplification. Inhibition of mTOR may also have effects on viral amplification, as CMV specifically upregulates the mTOR pathway during replication[26]. In allogeneic HCT and solid organ transplantation lower risk of CMV activation has been reported in patients treated with sirolimus [27,28].

iii. Antineoplastic effects. The mTOR signaling pathway is often constitutively activated in various human cancers. Sirolimus has been shown to induce cell cycle arrest *in-vitro* in both B-CLL and diffuse large B-cell lymphoma cells, and its efficacy as an antiangiogenic agent has been demonstrated in several experimental cancer models [29-33]. 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)[34].

2. Sirolimus for acute GVHD prophylaxis. Four clinical trials with sirolimus have been published from the Dana-Farber Cancer Institute [27,35-37]. 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 (myeloablative vs reduced intensity conditioning (fludarabine 120 mg/m^2 and busulfan 3.2 mg/kg)), donor source (related vs unrelated) and stem cell source (bone marrow vs. peripheral blood stem cells) (Table 1). In a retrospective study by Armand et al.[38] 190 patients undergoing transplantation for lymphoma were analyzed. The cohort consisted of patients treated with myeloablative (n=64) or reduced intensity conditioning (n=126) and transplanted with bone marrow (n=15), PBSC (n=168) or umbilical cord (n=7) grafts from either related (n=78) or unrelated donors (n=112). One-hundred-and-twenty six of the patients were treated with a combination of sirolimus with tacrolimus ± MTX, similar to the above mentioned Dana-Farber trials, while the remaining 90 patients received calcineurin inhibitor and MTX based immunosuppression. Although overall survival was superior in patients treated with sirolimus when the whole cohort was analyzed, multivariate analyses revealed that the benefit was restricted to patients undergoing reduced intensity conditioning (sirolimus group (n=103) 66% vs non-sirolimus (n=23) 38%; p=0.007) independently of MTX. In the same subgroup of patients sirolimus treatment was also associated with lower probability of progression (42% vs 74%, p=0.001), while no effect was observed on non-relapse mortality (sirolimus group 14% vs non-sirolimus group 9%, p=0.6). No statistically significant associations between sirolimus and the incidences of GVHD were observed (sirolimus-group vs non-sirolimus: acute GVHD: grade II-IV 14% vs 22%, p=0.6; grade III-IV 6% vs 13%, p=0.4, chronic GVHD 63% vs 48%, p=0.2). In a small study of 15 patients receiving primarily PBSC from 9 unrelated and 6 related fully HLA matched donors after reduced intensity conditioning (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)[39]. 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.[40], 23 patients with myelofibrosis were transplanted using a primarily fludarabine and melphalane based reduced intensity conditioning 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). Five out of six evaluable patients developed chronic GVHD in the CSP/MMF group, compared to nine out of 14 in the sirolimus/tacrolimus group. 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).

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Table 1. Summary of clinical trials

	Antin et al.[35]	Cutler et al.[27]	Cutler et al.[36]	Alyea et al.[37]	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.

E. 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, as well as for those patients previously transplanted with either syngeneic or allogeneic stem cells. Please see section 11.H (Conditioning Regimen) for criteria for 3 Gy TBI.

4. Proposal

The addition of sirolimus in protocol 1938 produced promising results in lowering grades 2-4 acute GVHD but did not achieve our goal of reducing grades 2-4 acute GVHD to less than 40%. However, the results using triple therapy with the addition of sirolimus to the "standard" immunosuppressive regimen was very promising and there was a significant reduction in acute GVHD. The goal of the current protocol is to compare in a phase III randomized trial, the effectiveness of 2 GVHD prophylaxis regimens in preventing acute grades II-IV GVHD. Each of the combinations will use CSP every 12 hours through day 96 then tapered off by day 150. Arm 1 (similar to the former unrelated donor nonmyeloablative protocol 1938 Arm 2) will be the "reference arm" in which MMF is given three times a day until day 30 then dropped to twice a day until day 40 with a taper from day 150 through day 180. In Arm 2, sirolimus will be given until day 150 with a taper though day 180. In Arm 2, in addition to CSP and sirolimus, MMF will be given three times a day until day 30, dropped to twice a day

and then discontinued on day 40. The current protocol's primary objective is to reduce the incidence of acute GVHD after unrelated nonmyeloablative HCT.

The specific design of the protocol is as follows. Patients with hematologic malignancies will be randomly assigned to one of two postgrafting immunosuppression schedules to determine which is the most effective at reducing the incidence of acute GVHD below the current incidence of 50%. Randomization is based upon transplant center (FHCRC vs other). We will study which of the treatment arms offers the best post-grafting immunosuppression regimen capable of achieving the primary objective of reducing the incidence of grades II-IV acute GVHD. Secondary outcomes will be 1) compare the non-relapse mortality, and 2) comparing overall and progression-free survivals in the 2 arms.

5. Primary Objective

1. To compare the effectiveness of 2 GVHD prophylaxis regimens in preventing acute grades II-IV GVHD.

6. Secondary Objectives

1. Compare non-relapse mortality in the 2 arms
2. Compare survival and progression-free survivals in the 2 arms.

7. Patient Selection

A. Inclusions

Ages >50 years with hematologic malignancies treatable by 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; or 4) Patients with a diagnosis of CLL (or small lymphocytic lymphoma) or diagnosis of CLL that progresses to prolymphocytic leukemia (PLL), or T-cell CLL or PLL.
- **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.

B. Exclusions:

1. Patients with rapidly progressive intermediate or high grade NHL.
2. Patients with a diagnosis of CMML.
3. Patients with RAEB who have not received myelosuppressive chemotherapy i.e. induction chemotherapy.
4. CNS involvement with disease refractory to intrathecal chemotherapy. For LP requirement, see Appendix N.
5. Presence of circulating leukemic blasts (in the peripheral blood) detected by standard pathology for patients with AML, ALL or CML.
6. Presence of ≥ 5% circulating leukemic blasts (in the peripheral blood) detected by standard pathology for patients with MDS/MPS
7. Fertile men or women unwilling to use contraceptive techniques during and for 12 months following treatment.
8. Females who are pregnant or breast-feeding.
9. Patients with active non-hematological malignancies (except non-melanoma skin cancers) or those with non-hematological malignancies (except non-melanoma skin cancers) who have been rendered with no evidence of disease, but have a greater than 20% chance of having disease recurrence within 5 years.
   This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy.
10. Fungal infections with radiological progression after receipt of amphotericin B or active triazole for greater than 1 month.

11. Organ dysfunction.
    a. Cardiac ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of < 26%). Ejection fraction is required if age > 50 years or there is a history of anthracycline exposure or history of cardiac disease. Patients with a shortening fraction < 26% may be enrolled if approved by a cardiologist.
    b. Pulmonary:
        i) DLCO < 40%, TLC <40%, FEV1 <40% and/or receiving supplementary continuous oxygen.
        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.
12. Karnofsky scores < 60 (see appendix B) or Lansky Score <50 (see appendix C).
13. Patient has poorly controlled hypertension and on multiple antihypertensives
14. HIV positive patients.
15. Active bacterial or fungal infections unresponsive to medical therapy.
16. All patients receiving antifungal therapy voriconazole, posaconazole, or fluconazole and who are then randomized to ARM 2 must have sirolimus reduced according to the Standard Practice Antifungal Therapy Guidelines in Appendix E.
17. 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. **FHCRC matching allowed will be Grades 1.0 to 2.1 (Appendix O)**: Unrelated donors who are prospectively:
    i) Matched for HLA-A, B, C, DRB1 and DQB1 by high resolution typing;
    ii) ***Only a single allele disparity*** will be allowed for HLA-A, B, or C as defined by high resolution typing (see **Appendix O for other donor selection details**).
2. Donors are excluded when preexisting immunoreactivity is identified that would jeopardize donor hematopoietic cell engraftment. This determination is based on the standard practice of the individual institution. The recommended procedure for patients with 10 of 10 HLA allele level (phenotypic) match is to obtain a panel reactive antibody (PRA) screens to class I and class II antigens for all patients before HCT. If the PRA shows >10% activity, then flow cytometric or B and T cell cytotoxic cross matches should be obtained. **The donor should be excluded if any of the cytotoxic cross match assays are positive**. For those patients with an HLA Class I allele mismatch, flow cytometric or B and T cell cytotoxic cross matches should be obtained regardless of the PRA results. A positive anti-donor cytotoxic crossmatch is an absolute donor exclusion.
3. Patient and donor pairs homozygous at a mismatched allele in the graft rejection vector are considered a two-allele mismatch, i.e., the patient is A*0101 and the donor is A*0102, and **this type of mismatch is not allowed.**

4. Only G-CSF mobilized PBSC 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.

9. Informed Consent

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

11. Plan of Treatment

A. Outline of treatment plan (refer to Figure 2 and Tables 2, and 3)

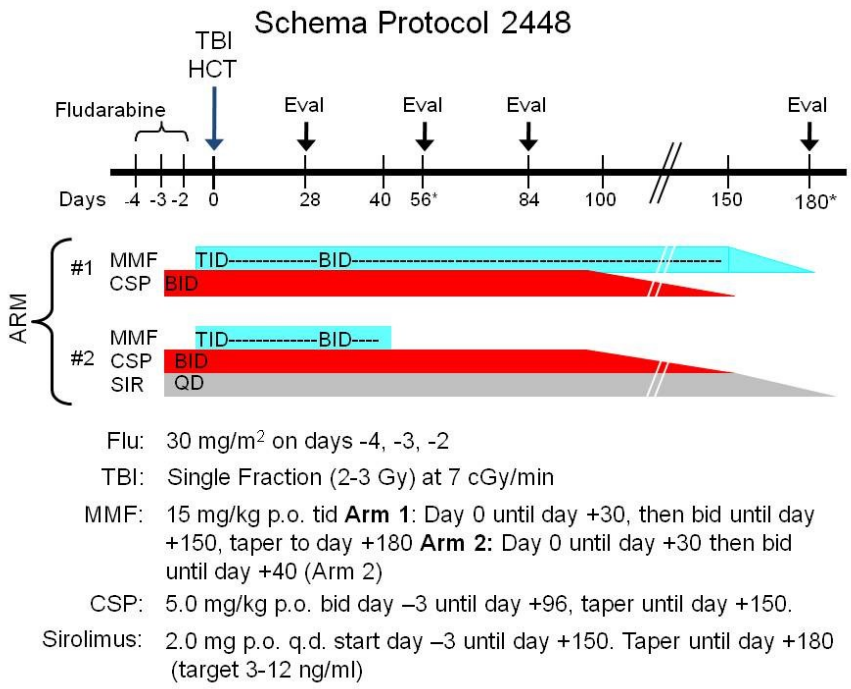


Figure 2. Protocol 2448 Schema.

If T-Cell (CD3) chimerism is **>50% on day +28**, then repeat only on days +84 and +365.
*If T-Cell (CD3) chimerism is **≤50% on day +28**, then repeat on days +56, +84, +180, & +365.
Granulocyte (CD33) chimerism on day +84 only.
Natural killer (NK) cell (CD56+) chimerism will be obtained on day +28.
Bone Marrow chimerism will be obtained on days +84 & +365
(See Patient Post-Transplant Evaluation for more details)

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

C. HCT: Transplant will be PBSCs collected as per NMDP standard. Twelve liter leukapheresis will be obtained on two consecutive days, and both collections will be infused on day "0". Because of logistical issues, PBSC infusions are usually performed in the hospital, but otherwise patients will only be admitted as medically necessary for control of transplant complications. Standard cryopreservation of a portion of PBSC will take place for potential DLI. A portion of the PBSC product will be removed for DLI that is equivalent to 3x10^7 CD3 cells/kg recipient weight and cryopreserved (see 11.I.1). If the product arrives after 6pm, 10% will be removed and held overnight prior to cryopreservation.

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

The need for this therapy should be discussed with the principal investigator. The referring oncologist may be asked to administer this therapy.

E. 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.

F. 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.

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

1. All patients with the diagnosis of CML or Ph+ ALL may continue treatment with imatinib mesylate, dasatinib or other BCR/ABL tyrosine kinase inhibitors until two days prior to HCT. The tyrosine kinase inhibitors should then be stopped to prevent possible inhibition of engraftment of donor stem cells.
2. **Imatinib mesylate or dasatinib and CNS prophylaxis and treatment:** For patients who require cranial-spinal irradiation, imatinib mesylate or dasatinib will need to be discontinued 48 hours prior to initiating cranial spinal irradiation. This discontinuation is necessary because the combined effects of cranial-spinal irradiation and imatinib mesylate or dasatinib on the CNS are not known.

H. Conditioning Regimen: (refer to Figure 2 and Tables 2 and 3)

1. Days -4, -3 and –2: Fludarabine 30 mg/m^2/day IV, administered over 30 minutes.
2. Day 0: TBI 2 or 3 Gy at 6-7 cGy/min from linear accelerator (Appendix U) followed by HCT. Regardless of the actual time of TBI administration on Day 0, immunosuppression should be given per schedule and prior to the infusion of PBSCs

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.

Table 2: Arm 1 - Conditioning Schema and Immunosuppression Schedule

Day Number	-4	-3	-2	-1	0	+1-29	+30-39	+40	+96	+150	+180
Fludarabine (30 mg/m²/day)	X	X	X								
TBI					2-3 Gy						
Stem Cell infusion					Infusion						
CSP (5.0 mg/kg q12hrs)		START	→	→	→	→	→	→	TAPER[A]	STOP	
MMF (15 mg/kg)					START[B] q8hr	→	Q12hr	→	→	TAPER[C]	STOP

[A] CSP should be tapered off over 55 days 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] MMF should be tapered off over 30 days in patients ***without*** preceding acute GVHD requiring therapy.

Table 3: Arm 2 - Conditioning Schema and Immunosuppression Schedule

Day Number	-4	-3	-2	-1	0	+1-29	+30-39	+40	+96	+150	+180
Fludarabine (30 mg/m²/day)	X	X	X								
TBI					2-3 Gy						
Stem Cell infusion					Infusion						
CSP (5.0 mg/kg q12hrs)		START	→	→	→	→	→	→	TAPER[A]	STOP	
MMF (15 mg/kg)					START[B] q8hr	→	Q12hr	STOP			
Sirolimus (2 mg QD)		START	→	→	→	→	→	→	→	TAPER[C]	STOP

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

3. **Immunosuppression**
   - Day –3. ***All patients:*** Commence cyclosporine at 5.0 mg/kg PO Q12 hours, continue to day +96 and then taper until day +150.
   - Day –-3. ***Arm 2 patients:*** Sirolimus daily dose of 2.0 mg PO QD, continue to day +150 and then taper until day +180
   - Day 0: ***Arm 1 and 2 patients:*** **After** HCT on day 0, mycophenolate mofetil (MMF) will be given based on adjusted body weight, at 15 mg/kg PO at 4-6 hours after SCT infusion is complete, then to be given at 15 mg/kg PO Q8 hours and then reduce to Q12 hours on day +30. **Arm 1:** Continue MMF Q12 hours until day +150 then taper until day +180. **Arm 2:** Continue MMF Q12 hours until day +40, MMF will then be discontinued without taper unless GVHD or disease relapse/progression occurs.

a. Cyclosporine (Arms 1 and 2 patients).

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. **For Arm 2 patients**, sirolimus should be given at least 4 hours after an oral dose of cyclosporine as concurrent administration leads to elevation of sirolimus levels.
2. Cyclosporine discontinuation:
   i. **Arms 1 and 2:** In the absence of acute or chronic GVHD, CSP is tapered at day 96 over 55 days (to be completed on Day +150).
   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 (and MMF) therapy are summarized below (section O).
3. Guidelines for CSP Dose Adjustment and Monitoring.
   i. Blood pressure, renal function (serum creatinine, BUN), electrolytes and magnesium need to be followed at least three times per week during the first month, twice weekly until day

+100, then once per week until CSP is stopped, unless clinical circumstances suggest the need for more frequent evaluations.

ii. CSP, whole blood "trough" levels (i.e., just prior to the next dose) will be evaluated on day 0 and twice weekly post-transplant until the initiation of the taper and adjusted if necessary to maintain blood levels that target upper end of therapeutic range (see Table 4) during the first 28 days. After taper, dose levels will be measured weekly if stable.

Table 4: **CSP Dose Adjustment**

	Patients on Arm 1		**Patients receiving sirolimus on Arm 2**	
	CSP Level to Target Using LC-MS/MS Method	**CSP Level to Target Using Immunoassay Method**	**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)	400 ng/ml	500 ng/ml (upper end therapeutic range for this method)	350 ng/ml	400 ng/ml (upper end therapeutic range for this method)
After Day +28	120 - 360 ng/ml	150 - 450 ng/ml	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	25% dose reduction	25% dose reduction
Patients on Hemodialysis	320 ng/ml	400 ng/ml	320 ng/ml	400 ng/ml

iii. **For Arm 2 patients** Do not exceed cyclosporine levels > 350 ng/mL to reduce risk of sirolimus toxicity.

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 between 120 and 360 will be targeted for patients on Arm 1, and between 120 and 300 for patients on Arm 2.

vi. Dose reductions should only be made if CSP toxicity is present or levels exceed upper limits of target by 20%, depending on method (see Table 4), in the absence of toxicity. 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

Decrease CSP levels	**Increase CSP levels**	**Enhance Potential for Nephrotoxicity**
Phenytoin	Erythromycin Diltiazem	Aminoglycosides
Phenobarbital	Alcohol Doxycycline	Loop diuretics (furosemide)
Carbamazepine	Ketoconazole Verapamil	Amphotericin formulations
Primidone	Acetazolamide 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 (Patients in arms 1 and 2)

1. Patients on Arms 1 and 2 will receive MMF
2. 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.

3. MMF discontinuation:
   i. **Arm 1:** MMF will be given daily at 15 mg/kg Q8 hours until **day +30**, at and then in the absence of GVHD, the dose will be changed to 15 mg/kg Q12 hours until **day 96.** In the absence of GVHD, MMF will be tapered at **day +150** by 25% per week with MMF discontinued after day + 180.
   ii. **Arm 2:** MMF will be given daily at 15 mg/kg Q8 hours until **day 30** post transplant, at and then in the absence of GVHD, the dose will be changed to 15 mg/kg Q12 hours until **day 40**. MMF will then be discontinued without taper unless GVHD or disease relapse/progression occurs
4. 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).
5. Guidelines for MMF dose adjustment due to drug toxicity:
   i. If in the clinical judgment of the investigator the observed toxicity is related to MMF administration, a dose adjustment may occur. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF).
   ii. Gastrointestinal Toxicity. Severe gastrointestinal toxicities such as gastrointestinal hemorrhage have been very rare after nonmyeloablative HCT. In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea that is considered to be due to MMF after day 28, a 20% dose reduction will be made or the drug may be given IV. If severe refractory diarrhea or overt gastrointestinal bleeding occurs, MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.
   iii. Neutropenia. Based on previous experience in patients after nonmyeloablative HCT, dose adjustments are likely to occur because of hematopoietic adverse effects, in particular neutropenia. A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for grade IV neutropenia that persists after day 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 (Arm 2 patients only)

1. Only patients on Arm 2 will receive sirolimus.
2. 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 [41-43]. 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) [41]. 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 [44].
   ii. **Patients with BSA > 1.5** m^2**:** Sirolimus will be started on day –3 at 2.0 mg every day orally through day 150. In the absence of GVHD, Sirolimus should be tapered at day 150 by 25% per week for 4 weeks and discontinued on day + 180. In the presence of GVHD or if the patient is receiving glucocorticoid therapy, continuation of sirolimus will be at the discretion of the attending physician or GVHD attending/team (see **14.G** GVHD treatment guidelines).
   iii. **Patients with BSA < 1.5** m^2**:** For 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.
3. 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 weekly starting on day 0, Mondays through Fridays only. Levels should also be drawn after changing the dose of sirolimus or adding any of the medications known to interfere with the sirolimus metabolism (Appendix R).

4. The dosage will be replaced if the patient vomits within 15 minutes of taking a dose. Premedication with clinically indicated antiemetics is acceptable if vomiting occurs.
5. If there is evidence of disease progression and no evidence of GVHD, patients will stop sirolimus and MMF without a taper (Arm 2 patients), 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 enrollment in other institutional protocols for DLI treatment.
6. 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.
7. Severe neutropenia or thrombocytopenia. The combination of sirolimus and cyclosporine interaction is that there is an increased risk of sirolimus toxicity such as anemia, diarrhea, hypokalemia, and thrombocytopenia. A thorough evaluation 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 infusions of Donor PBSC

1. G-CSF Administration to Donors: Timing of PBSC collection is prearranged through the NMDP. Day 0 should be fixed on a Monday-Thursday when possible. G-CSF will be administered by subcutaneous injection to the

unrelated donor starting 5 days prior to the day of HCT (see Table 6) as per NMDP protocol. Donors will receive approximately 10 μg/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.

a. Immunophenotyping of the PBSC product for the Seattle patients will be performed by the Cellular Therapy Laboratory and will include CD34, CD3/4 and CD3/8 cells. 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 unrelated donors will be frozen according to standard cryopreservation for DLI.

Table 6. Treatment Schema for Donor

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

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

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

J. ABO incompatibility

All patients with ABO incompatibility should be evaluated and treated as according to the standard practice of the individual institution. Recommendations are provided in Appendix 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 [46]. Therefore, these patients should be monitored and treated aggressively when there is any evidence of hemolysis.

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

L. Post-transplant Maintenance Therapy with Tyrosine kinase inhibitors (Imatinib, Dasatinib, etc.) for Ph (+) CML or 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>, the tyrosine kinase inhibitor therapy can be increased or other therapy considered (see section 11.O.6.e).

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

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

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

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

1. **<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 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 (Arms 1 and 2) and sirolimus (Arm 2) 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 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 unrelated HCT will be evaluated with the intermittent analysis of tumor markers: Responses will be classified as complete, partial response or no response. Response criteria for MM, NHL, CLL, CML, ALL, AML and MDS to be used in this study are described in Appendix 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.
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.

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

Additionally, see the following tables (Tables 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 12 and 13 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 12 and 13 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 12 and 13 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 ANC>500 for 2 days post nadir
4. Cyclosporine trough levels on day "0" and then twice a week until taper begins. Weekly thereafter if levels are stable.
5. Chem 1(Na+, K+, Cl_, Bun, Cr, glucose) and chem 3 (Mg+2, Ca+2) 3x per week until CSP taper begins.
6. FOR PATIENTS ON ARM 2 ONLY:
7. a) Sirolimus trough levels on day "0" and then twice a week for the first month and weekly thereafter to maintain therapeutic serum levels.

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

   c) Haptoglobin every other week until Day +56, then as indicated. Evaluation of schistocytes weekly with CBC through Day + 56.
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 unrelated HCT would be eligible for discharge. Since the patient may be discharged prior to starting CSP taper, instructions should be provided for preventing and detecting GVHD as per standard practice 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 IVIG monitoring and replacement therapy per Institutional Guidelines and patients with MM should be assessed for the need of bisphosphonates 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	x	x	x	x	x	x	x
	BM biopsy									
	Pathology	Clinical	*If abnormal pre-transplant			*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	PCR for bcr-abl, and p.210 breakpoint	Clinical	*If bone marrow not done and reflexive testing for 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				

2448.00

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 AND bone marrow not done	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	β-2 Microglobulin	Clinical				x				
	LDH	Clinical				x	x	x	x	x
	Imaging									
	CT of chest, abdomen, pelvis (neck if indicated)	Clinical	*If abnormal pre-transplant		*See comment	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

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

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
	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 2448"	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 2448"	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**

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

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

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

D. Sirolimus

1) Formulation and Administration

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

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

c. Sirolimus is to be administered orally once daily at the doses described in Section 11.H.3.c. To minimize variability of exposure to sirolimus, this drug should be taken consistently with or

without food. Grapefruit juice reduces CYP3A4-mediated metabolism of sirolimus and should not be administered with sirolimus or used for dilution.

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

2) Adverse Reactions

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

Appendix 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 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.3.b** for information about administration and dosage adjustments. *Mycophenolate mofetil (MMF)*: is supplied in 250mg hard gelatin capsules. Capsules

may be stored at room temperature.

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

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

G. GVHD:

1. Diagnosis: Skin involvement will be assessed by biopsy with percentage of body surface area involved recorded. GI symptoms suspicious for GVHD will be evaluated by biopsy as indicated. Acute GVHD and chronic GVHD will be graded according to established criteria (**Appendix F and G**). Acute GVHD will graded by an independent reviewer blinded to study arm and patient identity.
2. Recommended Treatment:
   a. Patients developing acute GVHD ≥ grade II off immunosuppression or while on a CSP taper:
      i. CSP 5mg/kg PO q12hrs. If there is concern of GI absorption use IV route (1.5mg/kg q12hrs).
      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:
      i. Patients who develop acute GVHD ≥ grade II should receive prednisone (1-2 mg/kg/day) or intravenous equivalent. Continuation of sirolimus (Arm 2) 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. **ALL Arms**: When steroids are tapered to less than 0.5 mg/kg, then a CSP taper should be initiated no sooner then day +96 and such that the completion of the taper is NOT prior to Day + 150.

- **Arm 1 Patients:** After successful discontinuation of CSP and corticosteroids, the suggested sequence for tapering MMF is to taper the MMF such that the completion of the taper is NOT prior to Day + 180 post transplant.
- **Arm 2 Patients:** If patient still receiving MMF, it should be discontinued without a taper prior to initiating a CSP taper. After successful discontinuation of MMF, CSP and corticosteroids, the suggested sequence for tapering sirolimus is to taper the sirolimus such that the completion of the taper is NOT prior to Day + 180 post transplant.
- If nausea and/or vomiting prevent the oral administration of CSP or MMF, then CSP and MMF should be administered intravenously. The timing of these tapers depends on the day post transplant that acute GVHD develops, the severity of the GVHD and the clinical discretion of the attending physician.
- Patients may be eligible for institutional trials of GVHD therapy

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

d. Patients off immunosuppression who develop concurrent manifestations of GVHD that satisfy criteria for acute GVHD $\geq$ 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 $\leq$500/uL and/or platelet count to $\leq$ 20,000/uL. If myelosuppression occurs, a bone marrow aspirate and biopsy should be considered to exclude disease progression. Samples should be sent for chimerism analysis by a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or FISH studies or VNTR) of the patient and donor. Myelosuppression may occur in this patient population for a number of reasons such as direct toxic effect of drugs (MMF, ganciclovir etc.), rejection, relapse or after DLI.

Patients with myelosuppression may be managed as follows:

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

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 Consideration and Termination of Study

This is a randomized phase III study whose primary objective is to compare two immunosuppressive regimens for their ability to prevent grades II-IV acute GVHD. Since the two arms differ in the number of drugs and the duration of administration, it is not feasible to blind the study; however, the assignment of GVHD grades will be performed by an independent evaluator who will be blinded as to the study arm.

A. Primary Endpoint

The primary endpoint for this trial will be the rate of acute grade II-IV GHVD at day 100 post-transplant, exclusive of GVHD that occurs as a result of alterations to immunosuppressive therapy in response to relapse or progression.

B. Sample size/Power

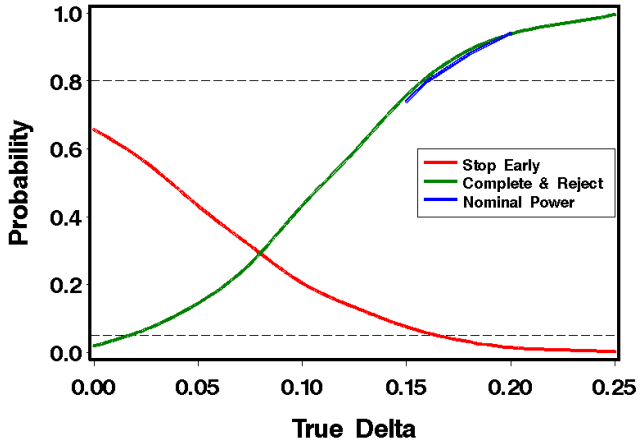
One hundred fifty patients will be randomized to each arm, for a total sample size of 300 patients, including patients who were randomized to the corresponding arms of the original 3-arm trial. The randomization will be stratified on institution. This sample size is sufficient to detect differences between arms in a range considered to be clinically significant – which is a difference of 15-20% percentage points. For such a difference the power ranges from 74% to 94%, at the 2-sided 0.05 level of significance, assuming a chi-squared test. For purposes of sample size analysis we have assumed a rate of 52% grade II-IV GVHD in one arm, which corresponds to our historical experience with MMF/CSP; however, the power calculations are not particularly sensitive to this rate. At a projected accrual rate of approximately 60 patients per year it will take 5 years to complete the study.

To better account for the competing risks of early death and relapse, the actual analysis will be a time-to-event analysis using a log-rank statistic. Patients dying or relapsing within the first 100 days post-transplant in the absence of acute GVHD will be censored at that time.

C. Interim Analysis for Futility

After 150 patients have been randomized and evaluated for 100 days post-transplant, an interim analysis for futility will be conducted. This analysis will estimate the conditional power of the study, given the data available at that time and assuming that going forward the rates of GVHD will differ by 15% between arms, with the rates for each arm centered around the pooled estimate of the rate from the combined arms.

The conditional power will be estimated by Monte Carlo simulation. If the estimated conditional power is <33.3% then the trial will be stopped, otherwise the trial will continue to completion. No adjustment to the final significance level is required. The overall properties of this rule are summarized in the figure as a function of the true difference (delta) between arms. The blue line indicates the nominal power of the study without the futility stopping rule. The green line indicates the probability of completing the trial and rejecting the hypothesis of equality at the 0.05 significance level. The red line indicates the probability of stopping the trial for futility. This rule preserves the nominal power of the study while still providing a reasonable likelihood of stopping the trial when the true difference between arms is clinically negligible.



D. Secondary Endpoints

Secondary endpoints that will be analyzed include grade III-IV acute GVHD, chronic extensive GVHD, non-relapse mortality, relapse, and overall survival. The small number of patients that were treated on the discontinued arm of the original protocol will be followed for primary and secondary endpoints, but will not be part of any comparative analysis.

17. Data and Safety Monitoring Plan

A. FHCRC Protocol 2448 Data and Safety Monitoring Plan

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

Protocol 2448 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 unrelated 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 an 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 2448 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 2448 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 IRBs that review the protocol and who 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 by the PI.

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

The adverse event reporting in this multi-institutional clinical trial will follow the FHCRC Guidelines for SAE reporting. These guidelines (attached in **appendix I**.) detail the expedited reporting requirements, definitions of particular events. All SAEs that meet expedited criteria are reported to the IRO within 10 days by the investigator, trial coordinator, or research nurse upon learning of the event. A completed SAE report form, signed by the PI, must be received by the IRO within 10 calendar days. The PI reviews all SAEs and annual reports at the time of submission. For patients being cared for at the 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 toxicities. 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

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

Ethnic Category	Gender		
	Females	Males	Total
Hispanic or Latino	4	5	9
Not Hispanic or Latino	123	168	291
Ethnic Category Total of All Subjects	127	173	300
Racial Categories			
American Indian / Alaska Native	1	2	3
Asian	3	4	7
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	3	5	8
White	120	162	282
Racial Categories: Total of All Subjects	127	173	300

19. References

Reference List

1. Niederwieser D, Maris M, Shizuru JA, et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood* 2003;101(4):1620-1629.
   Reference ID: 21841

2. Maris MB, Niederwieser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood* 2003;102(6):2021-2030.
   Reference ID: 23067

3. Maris MB, Sandmaier BM, Storer BE, et al. Unrelated donor granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing. *Biol Blood Marrow Transplant* 2006;12:454-465.
   Reference ID: 29095

4. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol* 2005;23(9):1993-2003.
   Reference ID: 25864

5. Nash RA, Antin J, Karanes C, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine (CSP) for prophylaxis of acute graft-versus-host-disease (GVHD) after marrow transplantation from unrelated donors [abstract]. *Blood*. 1997;90 (Suppl. 1)561a.
   Reference ID: 14922

6. Horowitz MM, Przepiorka D, Bartels P, et al. Tacrolimus vs. cyclosporine immunosuppression: results in advanced-stage disease compared with historical controls treated exclusively with cyclosporine. *Biol Blood Marrow Transplant* 1999;5:180-186.
   Reference ID: 16311

7. Yu C, Storb R, Deeg HJ, Schuening FG, Nash RA, Graham T. Synergism between mycophenolate mofetil and cyclosporine in preventing graft-versus-host disease in lethally irradiated dogs given DLA-nonidentical unrelated marrow grafts [abstract]. *Blood*. 1995;86 (Suppl 1)577a.
   Reference ID: 11655

8. Sandmaier BM, Storb R, Santos EB, et al. Allogeneic transplants of canine peripheral blood stem cells mobilized by recombinant canine hematopoietic growth factors. *Blood* 1996;87(8):3508-3513.
   Reference ID: 11289

9. Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood* 1997;89(8):3048-3054.
   Reference ID: 12941

10. Storb R, Raff RF, Appelbaum FR, et al. DLA-identical bone marrow grafts after low-dose total body irradiation: the effect of canine recombinant hematopoietic growth factors. *Blood* 1994;84:3558-3566.
    Reference ID: 9507

11. Baron F, Sandmaier BM, Storer BE, et al. Extended mycophenolate mofetil and shortened cyclosporine failed to reduce graft-versus-host disease after unrelated hematopoietic cell transplantation with nonmyeloablative conditioning. *Biol Blood Marrow Transplant* 2007;13:1041-1048.
    Reference ID: 32059

12. Shi Y, Sahai BM, Green DR. Cyclosporin A inhibits activation-induced cell death in T-cell hybridomas and thymocytes. *Nature* 1989;339:625-626.
Reference ID: 5723

13. Blaha P, Bigenzahn S, Koporc Z, et al. The influence of immunosuppressive drugs on tolerance induction through bone marrow transplantation with costimulation blockade [erratum appears in Blood. 2003 Sep 15;102(6):1950]. *Blood* 2003;101(7):2886-2893.
Reference ID: 25142

14. Chapuis AG, Paolo RG, D'Agostino C, et al. Effects of mycophenolic acid on human immunodeficiency virus infection in vitro and in vivo. *Nat Med* 2000;6(7):762-768.
Reference ID: 23162

15. Izeradjene K, Revillard JP. Apoptosis of superantigen-activated T cells induced by mycophenolate mofetil treatment. *Transplantation* 2001;71(1):118-125.
Reference ID: 21431

16. McSweeney P, Abhyankar S, Foran J, et al. Favorable early tranpslant outcomes using tacrolimus and mycophenolate mofetil for GVHD prevention after matched sibling allografting. *Blood* 2003;102 (Part 1)(11):714a, #2641-
Reference ID: 24069

17. Sehgal SN. Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression (Review) [reprint in Clin Biochem. 2006 May;39(5):484-9]. *Clin Biochem* 1998;31(5):335-340.
Reference ID: 37329

18. Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J Immunol* 2006;177(12):8338-8347.
Reference ID: 34729

19. Gao W, Lu Y, El Essawy B, Oukka M, Kuchroo VK, Strom TB. Contrasting effects of cyclosporine and rapamycin in de novo generation of alloantigen-specific regulatory T cells. *Am J Transplant* 2007;7(7):1722-1732.
Reference ID: 38844

20. Frank DA, Robertson MJ, Bonni A, Ritz J, Greenberg ME. Interleukin 2 signaling involves the phosphorylation of Stat proteins. *PNAS* 1995;92(17):7779-7783.
Reference ID: 38814

21. Zeiser R, Leveson-Gower DB, Zambricki EA, et al. Differential impact of mammalian target of rapamycin inhibition on CD4+CD25+Foxp3+ regulatory T cells compared with conventional CD4+ T cells. *Blood* 2008;111(1):453-462.
Reference ID: 34022

22. Hoffmann P, Ermann J, Edinger M, Fathman CG, Strober S. Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Exp Med* 2002;196(3):389-399.
Reference ID: 22725

23. Hanash AM, Levy RB. Donor CD4+CD25+ T cells promote engraftment and tolerance following MHC-mismatched hematopoietic cell transplantation. *Blood* 2005;105(4):1828-1836.
Reference ID: 38845

24. Edinger M, Hoffmann P, Ermann J, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med* 2003;9(9):1144-1150.
Reference ID: 31079

25. van de Laar L, Buitenhuis M, Wensveen FM, Janssen HL, Coffer PJ, Woltman AM. Human CD34-derived myeloid dendritic cell development requires intact phosphatidylinositol 3-kinase-protein kinase B-mammalian target of rapamycin signaling. *J Immunol* 2010;184(12):6600-6611.
Reference ID: 38816

26. Johnson RA, Wang X, Ma XL, Huong SM, Huang ES. Human cytomegalovirus up-regulates the phosphatidylinositol 3-kinase (PI3-K) pathway: inhibition of PI3-K activity inhibits viral replication and virus-induced signaling. *J Virol* 2001;75(13):6022-6032.
Reference ID: 38813

27. Cutler C, Kim HT, Hochberg E, et al. Sirolimus and tacrolimus without methotrexate as graft-versus-host disease prophylaxis after matched related donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2004;10(5):328-336.
Reference ID: 30176

28. Fortun J, Martin-Davila P, Pascual J, et al. Immunosuppressive therapy and infection after kidney transplantation. *Transplant Infectious Disease* 9999;prepublished online June 11, 2010; doi: 10.1111/j.1399-3062.2010.00526.x-
Reference ID: 38824

29. Decker T, Hipp S, Ringshausen I, et al. Rapamycin-induced G1 arrest in cycling B-CLL cells is associated with reduced expression of cyclin D3, cyclin E, cyclin A, and survivin. *Blood* 2003;101(1):278-285.
Reference ID: 38872

30. Wanner K, Hipp S, Oelsner M, et al. Mammalian target of rapamycin inhibition induces cell cycle arrest in diffuse large B cell lymphoma (DLBCL) cells and sensitises DLBCL cells to rituximab. *Br J Haematol* 2006;134(5):475-484.
Reference ID: 38871

31. Geissler EK, Schlitt HJ, Thomas G. mTOR, cancer and transplantation (Review). *Am J Transplant* 2008;8(11):2212-2218.
Reference ID: 38817

32. Koehl GE, Andrassy J, Guba M, et al. Rapamycin protects allografts from rejection while simultaneously attacking tumors in immunosuppressed mice. *Transplantation* 2004;77(9):1319-1326.
Reference ID: 38819

33. Guba M, von Breitenbuch P, Steinbauer M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002;8(2):128-135.
Reference ID: 38820

34. Kauffman HM, Cherikh WS, Cheng Y, Hanto DW, Kahan BD. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. *Transplantation* 2005;80(7):883-889.
Reference ID: 38818

35. Cutler C, Antin JH. Sirolimus for GVHD prophylaxis in allogeneic stem cell transplantation (Review). *Bone Marrow Transplant* 2004;34(6):471-476.
Reference ID: 38812

36. Cutler C, Li S, Ho VT, et al. Extended follow-up of methotrexate-free immunosuppression using sirolimus and tacrolimus in related and unrelated donor peripheral blood stem cell transplantation. *Blood* 2007;109(7):3108-3114.
Reference ID: 34740

37. Alyea EP, Li S, Kim HT, et al. Sirolimus, tacrolimus, and low-dose methotrexate as graft-versus-host disease prophylaxis in related and unrelated donor reduced-intensity conditioning allogeneic peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2008;14(8):920-926.
Reference ID: 35728

38. Armand P, Gannamaneni S, Kim HT, et al. Improved survival in lymphoma patients receiving sirolimus for graft-versus-host disease prophylaxis after allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning. *J Clin Oncol* 2008;26(35):5767-5774.
Reference ID: 37064

39. Schleuning M, Judith D, Jedlickova Z, et al. Calcineurin inhibitor-free GVHD prophylaxis with sirolimus, mycophenolate mofetil and ATG in Allo-SCT for leukemia patients with high relapse risk: an observational cohort study. *Bone Marrow Transplant* 2009;43(9):717-723.
Reference ID: 38811

40. Snyder DS, Palmer J, Gaal K, et al. Improved outcomes using tacrolimus/sirolimus for graft-versus-host disease prophylaxis with a reduced-intensity conditioning regimen for allogeneic hematopoietic cell transplant as treatment of myelofibrosis. *Biol Blood Marrow Transplant* 2010;16(2):281-286.
Reference ID: 38627

41. Kaplan B, Meier-Kriesche HU, Napoli KL, Kahan BD. The effects of relative timing of sirolimus and cyclosporine microemulsion formulation coadministration on the pharmacokinetics of each agent. *Clin Pharmacol Ther* 1998;63(1):48-53.
Reference ID: 38821

42. Ferron GM, Mishina EV, Zimmerman JJ, Jusko WJ. Population pharmacokinetics of sirolimus in kidney transplant patients. *Clin Pharmacol Ther* 1997;61(4):416-428.
Reference ID: 38822

43. Johnson EM, Zimmerman J, Duderstadt K, et al. A randomized, double-blind, placebo-controlled study of the safety, tolerance, and preliminary pharmacokinetics of ascending single doses of orally administered sirolimus (rapamycin) in stable renal transplant recipients. *Transplant Proc* 1996;28(2):987-
Reference ID: 38823

44. Kelly PA, Gruber SA, Behbod F, Kahan BD. Sirolimus, a new, potent immunosuppressive agent (Review). *Pharmacotherapy* 1997;17(6):1148-1156.
Reference ID: 34749

45. Marty FM, Lowry CM, Cutler CS, et al. Voriconazole and sirolimus coadministration after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006;12(5):552-559.
Reference ID: 35925

46. Bolan CD, Childs RW, Procter JL, Barrett AJ, Leitman SF. Massive immune haemolysis after allogeneic peripheral blood stem cell transplantation with minor ABO incompatibility. *Br J Haematol* 2001;112(3):787-795.
Reference ID: 19140

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APPENDIX A

ELIGIBILITY GUIDELINES FOR DONOR PBSC APHERESIS FOR TRANSFUSION

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

APPENDIX B

THE KARNOFSKY PERFORMANCE STATUS SCALE

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

APPENDIX C
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 PBMC products will only be 2-5% of the product volume before infusion, the small quantity may cause a hemolytic transfusion reaction. According to the 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 PBMC components should be RBC depleted by Starch Sedimentation (flowsheet below). *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*
Post transplant blood component support will be according to Standard Practice Guidelines.

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

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

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

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

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

MAJOR ABO INCOMPATIBLE

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

MINOR ABO INCOMPATIBLE

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

APPENDIX E
INFECTIOUS DISEASE GUIDELINES

Please note that the content of these PDFs is from the Fred Hutchinson Clinical Research Division Standard Practice Manual and does not contain research related procedures.

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

cmvdiseasetreatment.pdf

Antifungal Therapy Guidelines

antifungal_therapy.pdf

Pneumonia / Pneumocystis Carinii Prophylaxis

pneumocystisjiroveci.pdf

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

Antibiotic.prophylaxis-Encapsulated.pdf

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 (ichthyosis), nodules, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)*. The extent of skin involvement and the skin thickness score for patients with scleroderma needs to be recorded at the time of diagnosis, when changes in treatment are made and when assessing treatment response. Medical photos are also useful for assessing the extent of skin involvement and response to treatment.
Nails	*B. Ridging, onychodystrophy, onycholysis*
Hair	*Premature graying (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair*
Mouth	Dryness, burning, gingivitis, mucositis, striae, *dryness, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tightness around the mouth, sensitivity to acidic, strong flavors, heat or cold, tooth decay*
Eyes	*Dryness, burning, blurring, gritty eyes, photophobia, pain*
Vagina/vulva	*Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not induced by ovarian failure or other causes*
Liver	Jaundice and elevated liver function tests not due to other causes (see laboratory

	tests)
Lung	*Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis*
GI	Anorexia, nausea, vomiting, diarrhea, *malabsorption, dysphagia, odynophagia*
Myofascial	*Stiffness and tightness with restriction of movement, occasionally with swelling, pain, cramping, erythema and induration, most commonly affecting the forearms, wrists and hands, ankles, legs and feet, inability to extend the wrists without flexing the fingers or the elbows, contractures*
Muscle	*A. Proximal muscle weakness, cramping*
Skeletal	*Arthralgia of large proximal girdle joints and sometimes smaller joints*
Serosal	*Unexplained effusions involving the pleural, pericardial, or peritoneal cavities not due to venocclusive disease of the liver, cardiac insufficiency, malignancy, infection, GM-CSF toxicity or other causes*

C. Laboratory Testing and Diagnostic Indicators of Chronic GVHD

Eye	*Schirmer's test with a mean value ≤ 5 mm at 5 minutes, or values of 6-10 mm in patients who have sicca symptoms, or keratitis detected by slit lamp examination*
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase ≥2 x upper limit, of normal, AST or ALT >3 x upper limit of normal or total serum bilirubin ≥1.6)
Lung	*New obstructive lung defect defined as an FEV_1 <80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV_1/FVC by >12% within a period of less than 1 year, thought not to be caused by an infectious process, asthma or recurrent aspiration from the sinuses or from gastroesophageal reflux. In the absence of GVHD in any other organ, the diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage, evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scan of the lungs, or confirmation by thoracoscopic biopsy.*
Esophagus	*Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry*
Intestine	Endoscopic findings of mucosal edema and erythema or focal erosions with histological changes of apoptotic epithelial cells and crypt cell drop out. Patients with unresolved acute GVHD may have more severe intestinal mucosal lesions including ulcers and mucosal sloughing.
Muscle	*Elevated CPK or aldolase, EMG findings consistent with myositis with biopsy revealing no other etiological process*
Blood	Thrombocytopenia (usually 20,000-100,000/µl), eosinophilia (> 0.4 x 10^3/uL), hypogammaglobulinemia. Hypergammaglobulinemia and autoantibodies occur in some cases.

D. Guidelines for Treatment of Chronic GVHD after allogeneic HCT

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

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

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.

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

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

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

APPENDIX 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 (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.

Expedited Reporting Requirements

All adverse events (whether occurring on-site or off-site), which in the opinion of the principal investigator are (1) unexpected, and (2) related or possibly related to the research and (3) serious or suggests that the research places research participants or others at a greater risk of physical or

psychological harm than was previously known or recognized must be submitted to the IRB within ten (10) calendar days of becoming aware of the event.

Definitions

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

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

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

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

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

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

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

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

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 working 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 the start of any protocol intervention and day 100 during the study will be recorded on the CRF (**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 reported 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.

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 protocol 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

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 _____

Pharmaceutical product/medical treatment/procedure

☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

#2 _____

Pharmaceutical product/medical treatment/procedure

☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

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

Report Completed by: Date:

_____ _____

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

Does this study involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer)? ☐ yes ☐ no If yes and the activity involves the 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).

_____ Date: _____

Signature of Principal Investigator

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

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 **2448** 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, MD 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)*

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 Dose:
☐ TBI 2 Gy
OR
☐ TBI 3 Gy:
Signature of **Local** Principal Investigator**:** _____ Date: _____

Transplant Center: _____

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

FHCRC Use Only

Study UPN#: _____

Internal B#:_____

Randomization Arm

☐ Arm 1: CSP to day +96 and then tapered to day +150, MMF tapered at day +150 and discontinued after day +180.
☐ Arm 2: CSP to day +96 and then tapered to day +150 , MMF to be discontinued on day + 40, and Sirolimus to be tapered at day +150 and discontinued on day +180.

_____ _____
Signature **Date**

APPENDIX L cont'd

Protocol 2448 Eligibility

I) Inclusion Criteria:

1) Yes ☐ No ☐ Unrelated donors who are prospectively:
**FHCRC matching allowed will be Grades 1.0 to 2.1 (Appendix O).
i) Matched for HLA-A, B, C, DRB1 and DQB1 alleles by high resolution typing **AND**
ii) Only a single allele disparity will be allowed for HLA-A, B, or C as defined by high resolution typing (See appendix O for other donor selection details)

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

iii) Yes ☐ No ☐ Have a negative anti-donor cytotoxic crossmatch.
NA ☐ Cytotoxic crossmatch **not done as patient and donor are phenotypically identical by molecular methods.**

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

One of the following criteria questions (2-5) must be marked "Yes" for the patient to enter on 2448.

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

3) 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.

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

5) 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.

II) One of the following criteria questions (6-16) must be marked "Yes" for the patient to enter on 2448. (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.)

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

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

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

9) Yes ☐ No ☐ **CLL** – must have either 1) failed to meet NCI Working Group criteria for complete or partial response after therapy with a regimen containing FLU (or another nucleoside analog, e.g. 2-CDA, pentostatin) or experience disease relapse within 12 months after completing therapy with a regimen containing FLU (or another nucleoside analog); 2) failed FLU-CY-Rituximab (FCR) combination chemotherapy at any time point; or 3) have "17p deletion" cytogenetic abnormality. Patients should have received induction chemotherapy but could be transplanted in 1st CR; or 4) Patients with a diagnosis of CLL (or small lymphocytic lymphoma) or diagnosis of CLL that progresses to prolymphocytic leukemia (PLL), or T-cell CLL or PLL
Describe which inclusion is specific for this patient:_____
_____.

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

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

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

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

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

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

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

III) Exclusion criteria:

Each of the following questions must be marked "No" Or "NA" for the patient to enroll on 2448.

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

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

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

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

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

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

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

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

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

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

All patients receiving antifungal therapy voriconazole, posaconazole, or fluconazole and who are then randomized to ARM 2 must have sirolimus dosing reduced according to the Standard Practice Antifungal Therapy Guidelines in Appendix E.

PI Signature: _____ Date: _____

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

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

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

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

PI Signature: _____ **Date**:

Yes ☐ No ☐ **Pulmonary:** DLCO < 40%, 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.

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.

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

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

31) Yes ☐ No ☐ HIV positive patients.

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

Note – the HCT-Comorbidity score is: _____

☐ FHCRC Patients:

Signature of person completing form: _____ Date: _____

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

_____ Date: _____

Patient signed IRB approved consent form. Date: _____

IRB file number: _____ Date of IRB approval: _____

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

_____ Date: _____

OR

☐ Outside Center Patients:

Signature of person completing form: _____ Date: _____

Patient signed IRB approved consent form. Date: _____

IRB file number: _____ Date of IRB approval: _____

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

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

APPENDIX M
Core Case Report Forms

APPENDIX N

Intrathecal Therapy Administration

intrathecaltherapy-combined.pdf

Appendix O

HLA Testing of Donors and Recipients Prior to Hematopoietic Stem Cell Transplantation

hlatype.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

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

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

†One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft. EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Appendix 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, itraconazole, CSP, methylprednisolone, and tacrolimus may increase levels

Appendix S

Weight / Adjusted Body Weight for Drug Dosing

weight_for_drug_dosing.pdf

Appendix T

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 U

Radiotherapy Treatment Guidelines (FHCRC Protocol 2448.00)
Brenda M. Sandmaier, RSO Docket 2010-305

1.5 Total Body Irradiation

1.5.1 Patients
Every patient will receive total body irradiation as part of the preparatory regimen for stem cell transplantation.

1.5.1.1 Most Patients
The treatment dose regimen will be 200 cGy in one fraction. No lung shielding will be used for this TBI scheme.

1.5.1.2 Patients with certain diseases at greater risk of rejection, or patients previously transplanted with either syngeneic or allogeneic stem cells:

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 and have >5% donor CD3 chimerism,
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.

The treatment dose regimen will be 300 cGy in one fraction. No lung shielding will be used for this TBI scheme.

1.5.2 Equipment

1.5.2.1 Modality:
High-energy photons with energy ≥ 6MV photons should be utilized. Although there is no upper limit on the energy as long as the skin dose requirements can be met, it is recommended that 18MV or lower be used.

The selection of energy is determined by the dose uniformity criterion.

1.5.3 Target Volume
The total body will be treated including the head and feet in one field (except in certain circumstances). Care should be taken to ensure that the patient is entirely within the 90% isodose decrement line of the beam (i.e., not in the penumbra region of the beam).

1.5.4 Target Dose

The prescription point is defined as the point along the longitudinal axis of the patient at the midline at the level of the umbilicus (see **Point 5**, Section 1.5.4.1). No tissue inhomogeneity correction will be made in the calculation of dose to the prescription point. The absorbed dose along the patient's head to toe axis (line formed by the intersection of the midsagittal plane and the midcoronal plane) shall be within 10% of the prescribed dose. The dose at selected anatomical points shall be calculated and these calculations are to be submitted as part of the quality assurance. Measurements of patient dimensions needed for the calculation of the prescription dose will be made at the time of the simulation for lung blocks. Measurement and calculations of required monitor units necessary for each treatment will be performed for both the expected upright treatment position (AP-PA fields) and the reclining, lateral decubitus position (AP-PA fields). In the event the patient proves too ill to receive a fraction in the upright position, dose calculation will have been pre-calculated to permit treatment in the lateral decubitus position).

1.5.4.1 Prescription Point:

1. **Head (Point 1)**: this reference point is defined along the longitudinal axis of the skull at the greatest mid-separation (immediately superior to the nasal bridge). The depth should be taken as midway between the entrance and exit points of the opposed radiation beams.
2. **Neck (Point 2)**: this reference point is defined along the patient's longitudinal axis at the level of C3/C4 (approximate mid-neck, but chosen for the thinnest mid-separation of the neck). The point is taken to be midway between the entrance and exit point of the beam.
3. **Upper Mediastinum (Point 3)**: this reference point is defined along the patient's longitudinal axis at the level of the angle of Louis. The reference point is midway between the entrance and the exit points of the opposed beams.
4. **Lower Mediastinum (Point 4)**: this reference point is defined along the patient's longitudinal axis at the level of the xiphisternal notch. The reference point is midway between the entrance and exit points of the opposed beams
5. **Umbilicus (Point 5)**: THE PRESCRIPTION POINT is defined along the patient's longitudinal axis at the level of the umbilicus. The prescription point is midway between the entrance and exit points of the opposed beams.
6. **Knee (Point 6):** this reference point is defined along the midline in the midplane of the knee at the level of the patella.
7. **Ankle (Point 7)**: this reference point is defined along the midline at the midplane of the ankle at the level of the lateral malleolus.
8. **Shielded Lung Dose (Point 8)**: this reference point is located on the right chest wall under the lung block. It is centered (both medial/lateral and cephalocaudad) under the lung block as projected on the patient's skin. The depth should be taken as midway between the entrance and exit points of the opposed radiation beams. Dose measurements at this location will be taken during a fraction with lung shielding in place.
9. **Unshielded Lung Dose (Point 9)**: This reference point is the same as point 8. Dose measurements at this location will be taken during a fraction without lung shielding in place. The depth should be taken as midway between the entrance and exit points of the opposed radiation beams.

1.5.4.2 Dose Definition:
The absorbed dose is specified as centigray (cGy)-to-muscle.

1.5.4.3 Prescribed Dose, and Fractionation, and Timing: Recommended Fractionation Scheme

1.5.4.3.1 Most Patients
The total dose to the prescription point shall be 200 cGy given in a single fraction**.** All fields should be treated each fraction.

1.5.4.3.2 Patients at greater risk of rejection
The total dose to the prescription point shall be 300 cGy given in a single fraction. All fields should be treated each fraction.

1.5.4.4 Dose Rate:
A mid-plane dose rate of between 6 and 15 cGy per minute is required.

1.5.4.5 Dose Uniformity:
The objective is to keep the dose throughout the body, defined to extend to within 2 mm of the skin surface, to at least 90% of the prescription dose. In addition, the brain dose shall not exceed 107% of the prescription dose.

For AP/PA treatments, partial transmission lung blocks will be used to limit the overall total lung dose. The dose at the midpoint of the thickest part of the body while in the treatment position should be determined and if necessary, modifications made to the treatment to raise the dose in this region to at least 90% of the prescription dose.

In order to satisfy the requirement that the skin dose at a depth of 2 mm is within at least 90% of the prescription dose, beam spoilers or other equally effective devices should be used. The field size shall be such that no part of the patient extends into the portion of the penumbra region where the dose is less than 90% of the central axis dose.

1.5.5 Treatment Technique
Patients will be treated using AP/PA fields in an upright seated or standing position in a TBI positioning device. Treatment will be delivered with equally weighted parallel opposed portals, with each treatment including both AP and PA fields. If the patient is unable to tolerate the upright position, acceptable alternate arrangements will include equally weighted AP-PA parallel opposed fields delivered to the patient in a lateral decubitus position on a treatment couch or gurney.

Changes in patient positioning after the patient has started TBI are discouraged. When unavoidable, to ensure compliance to the overall dose and lung shielding parameters, appropriate changes in lung blocking and dose recalculation will be required.

Young patients requiring anesthesia will be treated in an AP/PA configuration at extended distance. If more than a single field is needed to accomplish treatment, the field junction should occur at the level of the thighs and be shifted every 2 fractions.

1.5.5.1 Dose Calculation for the Prescription Point
The calculation of the treatment time or the monitor units for the prescribed dose can be carried out using standard techniques. However, TBI presents special problems relative to the routine treatment situation in that the field sizes are much larger and the treatment distances much longer. The TBI percent depth dose (PDD) or Tissue Maximum Ratio (TMR) and output factors should be measured under TBI treatment conditions for a range of phantom sizes to establish the database for TBI beam-on time calculations or to validate the calculation methodology.

Typically, a calculation methodology will be adopted which uses PDD or TMR and output factors measured under standard conditions but then modified to account for the larger treatment distance. For example, modified values for inverse square corrected percentage depth dose or tissue-air ratios and tissue-phantom ratios are necessary for some treatment units when the patient is positioned at a long distance from the photon source and near the floor or one wall of the room. Also, some deviation from an exact inverse square decrease with distance has been demonstrated for certain geometries.

Measurements of dose at the center of a phantom about the size of the typical patient should be performed and compared to the calculated dose. If differences are found, additional correction factors should be introduced to the calculation method.

1.5.5.2 Critical Organ Dose Points
The required dose calculations should be performed for the 9 points referenced above (1.5.4.1). The midline dose at these locations should be recorded on the TBI Summary Form.

The dose can be calculated based on the thickness at each location and factors appropriate to the TBI treatment conditions.

It is recommended that entrance and exit TLDs or diodes be placed on the patient at each required dose assessment location. The midline dose can be calculated from these measurements making the appropriate corrections to the readings and then averaging the corrected values.

In younger patients it is also recommended that TLDs or diodes be placed underneath the lung blocks to document the transmission dose and scatter dose.

1.6 Lung Shielding
No Lung shielding will be used

1.7. QA Documentation for Total Body Irradiation

1.7.1. Questions regarding the radiotherapy section of this protocol should be directed to the radiation oncology study coordinator:

Ralph Ermoian, MD,
University of Washington Cancer Center
Department of Radiation Oncology
1959 NE Pacific St.
Box 356043
Seattle, WA 98195
Work: (206)598-4100
Fax: (206)598-3786
Email: ralphpe@uw.edu

1.7.2 Definitions Of Deviation In Protocol Performance

Prescription Dose

Minor Deviation:

The dose to the prescription point differs from that in the protocol by between 6% and 10%.

Major Deviation:

The dose to the prescription point differs from that in the protocol by more than 10%.

Dose Uniformity

Minor Deviation:

The dose to any of the reference points in Section 16.5.5.1 differs from the protocol dose by more than 10% but less than 20%.

Major Deviation:

The dose to any of the reference points in Section 16.5.5.1 differs from the protocol dose by more than 20%.