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Nonmyeloablative Conditioning with Pre- and Post-Transplant Rituximab followed by Related or Unrelated Donor Hematopoietic Cell Transplantation for Patients with Advanced Chronic Lymphocytic Leukemia: A Multi-Center Trial

Principal Investigators: **Fred Hutchinson Cancer Research Center**

Mohamed L. Sorror, MD	Associate Member, FHCRC, Associate Professor of Medicine, UW	667-2765
David G. Maloney, MD	Member, FHCRC, Professor of Medicine, UW,	667-5616
Brenda M. Sandmaier, MD	Member, FHCRC, Professor of Medicine, UW,	667-4961
Stanley Riddell, MD	Member, FHCRC, Professor of Medicine, UW	667-5249
Mary E.D. Flowers, MD	Member, FHCRC, Professor of Medicine, UW	667-5115
Rainer Storb, MD	Member, FHCRC, Professor of Medicine UW	667-4407

Statistician: Barry Storer, Ph.D., Affiliated Associate Professor of Biostatistics, UW, Member, FHCRC (667-6533).

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

Emergency (24 hour) Phone: (206) 598-8902 **Fax:** (206) 598-4034

Additional Performance Sites	Investigators	Emergency Phone Numbers
Rigshospitalet	Niels Andersen, MD	+45 35 45 1134
City Hlth & Sci - Torino	Benedetto Bruno, MD	011-39-0339-1129064
Veterans Affairs Puget Sound Health Care System	Thomas Chauncey M.D., PhD	(206) 762-1010 for the oncologist on-call
Mayo Clinic	William Hogan, MD	(507) 284-2511

1. Introduction

Chronic lymphocytic leukemia (CLL) is a proliferative disorder of functionally abnormal lymphocytes. CLL is grouped with a spectrum of diseases known as low-grade lymphoproliferative disorders. CLL is the most common form of leukemia (25% of all cases) in western countries, and 95% of all cases are of B-cell phenotype. [1,2] Median age at diagnosis is 70 years, and only 10-15% of patients are younger than 50 years. [3] Despite some therapeutic progress, standard treatment is not curative for CLL. This, together with the advanced age of most patients and the relatively indolent course of the disease for some patients, makes symptom palliation a reasonable treatment goal. Therefore, chlorambucil or fludarabine are usually given for CLL patients who have anemia, thrombocytopenia, hepatosplenomegaly, and/or lymphadenopathy. Even though fludarabine was shown to result in higher response rates and longer progression free survival (PFS) compared to single-agent or combination chemotherapy, overall survival (OS) was not improved. [4-6] The use of fludarabine-rituximab or fludarabine-cyclophosphamide-rituximab combinations may improve PFS and appears to improve OS based on historical controls[7,8]. Despite these advances, most patients who live long enough eventually fail fludarabine therapy, and about 20% of CLL patients have primary refractoriness (defined by failing to meet NCI Working Group Criteria for complete or partial responses: see Appendix H). Fludarabine-refractory CLL patients have a poor prognosis with median survival of 12 months. [9,10] Re-treatment with fludarabine or other nucleoside analogues such as cladribine or pentostatin, combination chemotherapy with or without fludarabine, and new biological agents such as Rituximab and Campath-1H has not demonstrably improved PFS. [9,11-17]

HCT using myeloablative conditioning has been increasingly used to treat young patients with CLL who have early stage disease and limited prior treatment. [18] Autologous high-dose HCT for CLL has shown long-term remissions and transplant-related mortality (TRM) of less than 10%. However, this treatment option was associated with high relapse rates reaching 56%[19]. Allogeneic HCT with high-dose conditioning has the theoretic advantage of graft-versus-leukemia (GVL) effects, and has resulted in tumor eradication and lower relapse rates compared to autologous HCT. However, TRM is higher than after autologous HCT by 20-40%. Unrelated-donor HCT has not been widely used for treatment of CLL because of the higher TRM as compared to related-donor HCT[20-22]. Hence, there has been no standard curative therapy for patients with fludarabine-refractory CLL. The Cancer Therapy Evaluation Program (CTEP) at the National Institute of Health (NIH) has recommended clinical trials for patients who have failed fludarabine treatment.

Allogeneic HCT after nonmyeloablative conditioning with fludarabine and low dose total body irradiation (TBI) and postgrafting immunosuppression with cyclosporine (CSP) and mycophenolate mofetil (MMF) was developed in an attempt to eradicate hematologic malignancies by GVL effects while avoiding morbidities and mortalities associated with myeloablative HCT. This approach using unrelated donor grafts has resulted in an encouraging OS and PFS rates of 67% at 2 years in 16 patients with fludarabine-refractory CLL. [23] Our updated results with longer follow-up show OS and PFS of 50% and 39%, respectively, at 5-years, which exceed any conventional salvage therapy for patients with fludarabine-refractory CLL. [24] However, disease relapse remain a cause of treatment failure with 5-years rate of 38%. NRM was the other cause of treatment failure (23% at 5-years), which resulted mainly from GVHD and infections. Outcomes were comparable between related and unrelated recipients.

The current protocol will attempt to better define safety and efficacy of allogeneic HCT after nonmyeloablative conditioning as salvage therapy for high-risk CLL patients. We propose to add the chimeric monoclonal anti-CD20 antibody Rituximab once immediately prior and three times in the weeks following the current non-myeloablative conditioning regimen. Our main goal is to reduce disease relapse. We will collect pharmacokinetic data to better define the optimal dosing of Rituximab and its impact on response. In addition, we will attempt to identify the recipient/donor pairs that benefit the most from Rituximab. This will be done by assessing recipient and donor polymorphisms of the FCγRIIIa receptor, which have been shown in some series to affect responsiveness to Rituximab.

2. Background

A. Diagnosis, epidemiology, staging and prognosis of CLL

CLL is characterized by a progressive accumulation of atypical monoclonal lymphocytes in the bone marrow, peripheral blood and other organs. CLL is part of a spectrum of diseases grouped as low-grade lymphoproliferative disorders. It is the most common form of leukemia in western countries and constitute 25% of all cases. [1] The current annual incidence estimates vary from approximately 8100 to 12,500 new cases in the United States, [25,26] with annual CLL-related deaths of 4500. [27] The National Cancer Institute-sponsored Working Group (NCI-WG) on CLL had revised their guidelines for diagnosis of CLL. [28] All patients must meet all the following criteria to be diagnosed as CLL:

- An absolute peripheral lymphocytosis greater than 5,000 mature-appearing lymphocytes/μl.
- One or more B-cell markers (CD19, CD20, CD23) plus CD5 by flow cytometry.
- Mature lymphocytes with less than 55% cells being atypical lymphocytes, prolymphocytes or lymphoblasts.
- A normal to hypercellular bone marrow aspirate with ≥ 30% of the nucleated cells being of lymphoid origin.

Patients with CLL are staged utilizing either the Rai or Binet systems. Both systems discriminate CLL based on the sites of disease and/or degree of cytopenias induced by leukemic marrow replacement after exclusion of autoimmune etiologies. While, the Binet classification divides CLL patients into three stages as noted in, the Rai classification originally defined five stages of CLL (See Appendix R). However, in 1987, a modified Rai system was developed creating three prognostic risk categories (low, intermediate, and high risk) equivalent to the three stages of the Binet system. Factors predictive of poor prognosis in CLL patients include older age, male gender, a diffuse bone marrow pattern, a lymphocyte doubling time of less than one year, serum levels of B2-microglobulin greater than 4.0 mg/L, serum thymidine kinase levels greater than 7.0 U/L, and higher serum soluble CD23 levels. [29-32] Chromosomal analysis can also be used to assign risk. Patients with a normal karyotype, or isolated 13q14 deletions have a benign course. [33] However, 17p13 or 11q23 deletions (10-15% of patients) are associated with very poor prognosis. [34,35]

CLL has been categorized into two prognostic subsets based on the immunoglobulin variable-heavy chain (IgV_H) mutational status. It was found that Binet stage A CLL patients with somatically unmutated IgV_H genes have a median survival of 8 years compared to 25 years in patients with mutated genes. [36,37] Further, the two IgV_H subsets are distinct and do not metamorphose from one into the other. Although surface CD38 expression was originally suggested as a surrogate assay for V gene mutations, [36] it appears that there is a 30% discordance between the assays. [38] Furthermore, CD38 expression was found to change in 25% of cases during the course of the disease, and therefore was not a useful prognostic indicator. [38] More recently, zeta-chain-associated protein 70 (ZAP-70), a kinase linked to T-cell receptor activation, was shown to be as excellent surrogate marker for the V gene mutational status with a sensitivity and specificity of 91% and 100%, respectively. [39] Unlike CD38, ZAP-70 surface expression does not change over time. [39] Given the difficulty in performing IgV_H sequencing in a routine diagnostic laboratory, ZAP-70 detection with flow cytometry can be utilized efficiently in determining the prognosis of CLL patients or ensuring molecular complete remission (CR) after clinical trials.

B. Fludarabine-refractory CLL

Fludarabine has been considered frontline therapy for symptomatic CLL. Three large phase III trials of fludarabine in symptomatic untreated CLL patients have shown a higher response rate with prolongation of PFS compared to chlorambucil or combination chemotherapy. [5,6,40] There was no survival advantage in any of these studies likely due to crossover to fludarabine in the other arms. All surviving eventually failed fludarabine therapy, while approximately 20% of patients were refractory to primary therapy. Patients with fludarabine-refractory CLL have a very poor prognosis with a median survival of one year and less than 5% have responded to re-treatment with single-agent fludarabine. [9,41,42] These patients have had limited treatment options, and most investigators encourage their participation in clinical trials. [43-45] Several clinical trials have been performed on patients with fludarabine-refractory CLL using other nucleoside analogs like cladribine or pentostatin, combination chemotherapy, and monoclonal antibodies such as Rituximab or Campath-1H with limited improvement in disease-free survival and OS. In the current protocol, fludarabine-refractory CLL will be defined by failure to meet NCI-WG Criteria (Appendix H) for complete or partial response after a regimen containing fludarabine (or other nucleoside analogue) or relapse /progression within 12 months after completion of a regimen containing fludarabine (or other nucleoside analogue such as pentostatin or cladribine).

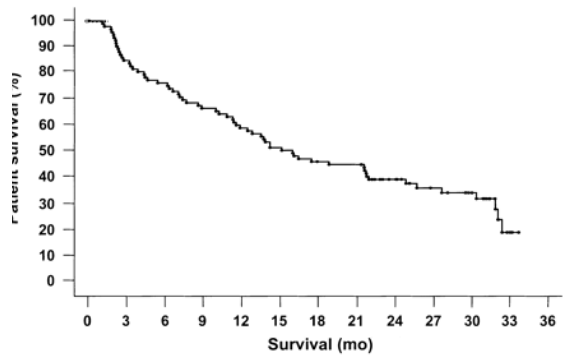


Figure 1. OS curve for patients with fludarabine-refractory CLL treated with Campath-1H.

The best clinical outcomes for patients with fludarabine-refractory CLL were associated with the use of Campath-1H. [14,17,46] Campath-1H is a humanized anti-CD52 monoclonal antibody that has been approved by the United States Food and Drug administration for treatment of fludarabine-refractory CLL (reviewed in [47]). Ninety-three CLL patients who were refractory to fludarabine were given Campath-1H in a prospective phase II trial involving 21 centers worldwide. The overall response rate was 33% however, only 2% achieved CR. Median time to progression was

4.7 months and median survival was 16 months. OS was approximately 45% at 18 months (Figure 1). [14] Since this was the best reported outcome for salvage therapy for fludarabine-refractory CLL patients, we will use this outcome as the gold standard for comparison in the current protocol. Campath-1H caused substantial toxicities that included infusion-related fevers and chills, infections in 55% of patients (25 of 93 patients had grade III-IV infections), and hematological toxicity (21% had grade IV neutropenia). Updated results in 152 fludarabine-refractory CLL patients who received CAMPATH-H1 showed 42.5% overall response rate and CR rate of 5%.[17] This update included 29 patients with T-cell prolymphocytic leukemia (T-PLL) who showed lower overall response rate of 24%, but 14% were in CR. CMV disease occurred in 4 patients and 5 patients died from infections. Duration of response and OS rate were not available in this update.
Rituximab which is a chimeric human-mouse monoclonal antibody directed against the CD20 antigen present on B cells, has been investigated in the treatment of fludarabine-refractory CLL. A trial of thrice-weekly dosing of rituximab in 33 patients with CLL/small lymphocytic lymphoma (SLL), 52% of whom were fludarabine-refractory, the overall response rate among the fludarabine-refractory subset was 41% with a median response duration of 6 months. [11] A dose-escalation study of 40 patients with CLL showed that only 20% of fludarabine-refractory patients achieved PR and none CR. In a multiple parameter analysis, sensitivity to fludarabine was the only factor that correlated significantly with response to rituximab[12], a finding that reflects the limited activity of rituximab as a single agent for treating fludarabine-refractory CLL patients.

Combination chemotherapy with nucleoside analogs has not resulted in better outcomes than that of Campath-1H. Twenty-eight patients with fludarabine-refractory CLL were treated with a combination of cyclophosphamide and fludarabine[9]. Overall response rate was 39% with 3% of patients achieving CR. Median survival was 12 months with OS rate of 30% at 18 months. Thirteen patients with fludarabine-refractory CLL or SLL were treated with pentostatin plus cyclophosphamide resulting in an overall response rate of 77% with only one patient achieving CR, but responses were transient and median survival was only 16 months[15]. Cladribine has shown modest clinical activity and considerable toxicity. In one study, cladribine was given alone to treat 28 fludarabine-refractory CLL patients who had modest fludarabine or chemotherapy exposure and lacked pre-treatment neutropenia and thrombocytopenia. Despite this selection, Overall response rate was 32% without achieving CR with median PFS of 9 months. Grades III-IV toxicities were frequent and included infections (43%), neutropenia (75%), and thrombocytopenia (68%)[13]. Another trial explored a combination of cladribine and cyclophosphamide in 16 purine analog-refractory patients. The overall response rate was 25% and only one patient achieved CR. Toxicities were significant with myelosuppression being the dose limiting toxicity[16]. A combination of fludarabine, cyclophosphamide, and rituximab was used to treat 102 CLL patients of whom 27 were refractory to fludarabine[48]. Overall response rate among the fludarabine-refractory patients was 59% with CR rate of 7%. Survival data were not reported in this study

C. Autologous HCT for CLL

Autologous HCT has been offered only to selected groups of patients with CLL. The main criteria of eligibility for autologous HCT were documented chemosensitivity, minimal tumor burden (typically requiring several cycles of cytoreductive chemotherapy), and successful collection of enough hematopoietic progenitor cells. Best results have been achieved in patients undergoing autologous HCT earlier in their disease course and in those with fewer previous treatments or relapses. [49] Concerns about studies investigating autologous HCT for CLL include 1) the limited applicability due to the judicious selection of patients likely to benefit from the approach, 2) the high probability of disease relapse, and 3) the long-term risk of treatment-related secondary malignancies (reviewed in[50]). Failure to respond to pretransplant debulking chemotherapy has been one of the difficulties with autologous HCT. [51] In a series of 20 CLL patients, 10 patients did not meet the eligibility criteria either because of chemotherapy resistance (n=5, 4 died from progression), mortality from chemotherapy complications (n=2), or failure to mobilize adequate numbers of C34+cells (n=3). [52] Others have described poor hematopoietic cell collection due to advanced disease stage[49,53], or from prior marrow toxic chemotherapy including fludarabine and chlorambucil[54,55]. Available purging methods were found to either have no impact on survival[56] or increase the risk of life threatening infections[57], thereby causing morbidity and mortality[58].
Two large studies using autologous HCT for CLL, one from the European Group for Blood and Marrow Transplantation (EBMT) and one from the International Project for CLL are summarized in Table.1.

Table1. Autologous HCT for CLL

	EBMT[59] (n=482)	International project for CLL[19] (n=124)
Age, median (range), years	50 (22-66)	49 (22-64)
Median interval from diagnosis to HCT (range), months	26 (4-215)	36 (5-218)
Prior regimens	50% received <3 regimens	*
Prior fludarabine exposure	26%	*
Chemo-sensitive disease at time of HCT	85% (CR=31%)	73%
Overall disease response	(n=239) 87% (CR=78%)	*
TRM	11% at 3-years	6% at 3 months
Relapse	41% at 3-years	58% at 5 years
DFS	*	32% at 5 years
OS	79% at 3-years, no plateau	63% at 5 years
Factors predicting better outcome	Early HCT (<36 months from diagnosis), low number of prior regimens, and being in CR at HCT	Early stage (Rai 0), short interval between diagnosis and HCT (<36 months0, low marrow involvement, and <1 prior regimens

* Not reported

The German CLL Study group treated 65 patients, median age of 49 years, with TBI/cyclophosphamide followed by transplantation of immunomagnetically purged autologous stem cells[60]. Median interval from diagnosis to HCT was 14 months, and 94% of patients were chemotherapy naïve. Protocol failures before HCT occurred in 25% of patients either because they were not in remission (n=7), had poor mobilization (n=3), and/or protocol violations (n=4). One-year DFS was 95% by clinical and 69% by molecular criteria. The same authors have shown that persistence of the leukemic clone was found among 24 of 30 studied patients (80%) by sensitive Taqman CDR3 amplification for clone-specific primers {Dreger P, von Neuhoff, et al. 2000 23081 /id}. The German CLL Study concluded that autologous HCT does not appear to cure CLL even if performed early.

The Fred Hutchinson Cancer Research Center (FHCRC) has enrolled 7 patients under protocol 962 for autologous HCT. All had advanced stage CLL and had failed primary therapy. Their median age was 57 years. Only 5 patients were able to receive their HCT. Two patients died after HCT, one from respiratory failure secondary to acute pneumonitis with pseudomonas septicemia 4 months after HCT and the other after receiving a second HCT for secondary AML 39 months after the initial HCT. Three patients are alive, 2 of whom have relapsed 50 and 52 months after HCT, and one of whom developed Hodgkin's disease 69 months after HCT.

In summary, autologous HCT has remained restricted to younger patients without heavy prior chemotherapy regimens and with early stage chemo-responsive disease with fewer numbers of prior regimens. Although autologous HCT resulted in relatively low TRM, has not been shown to eradicate CLL on the molecular level. Therefore, patients had remained at risk of relapse, and there has been no plateau in DFS or OS. Autologous HCT has never been studied in patients with fludarabine-refractory CLL.

D. Allogeneic HCT for CLL

Allogeneic HCT for CLL has been characterized by high TRM and low relapse incidence. The risk for TRM has been higher for older patients and those who have received unrelated donor grafts. Most reported studies using myeloablative allogeneic HCT for CLL focused on younger patients with good performance status and limited numbers of preceding chemotherapy regimens.

Despite the low relapse rate, survival after allo-HCT has been lower than after autologous HCT mainly because of high TRM up to 50% even in experienced centers[61]. The majority of allotransplants have used related donors, and the experience with unrelated donors is limited (reviewed in[62]).

Table 2 summarizes data reported from 4 large studies investigating the role of allogeneic myeloablative HCT from related or unrelated donor for treatment of CLL.

Table 2. Allogeneic myeloablative HCT for CLL

	International project for CLL[19] (n=48)	Nebraska and Tennessee[63] (n=23)	EBMT[59] (n=209)	CLL working group-NMDP[64] (n=40)
Donor Related Unrelated	 100% 0%	 87% 13%	 96% 4%	 0% 100%
Age, Median (range), years	43 (28-60)	46 (29-60)	47 (22-64)	44 (26-57)
Interval between diagnosis and HCT, median (range), months	26 (5-148)	19 (4-160)	45 (5-198)	45 (8-121)
Number of chemotherapy regimens	*	Median (range): 2 (1-6)	73% received <3 regimens	Median (range): 3 (0-5)
Chemotherapy sensitivity	57% refractory	61% refractory	56% chemo-responsive	50% chemo-refractory
Fludarabine exposure	*	65% received prior FLU	21% received prior FLU	80% received prior FLU
Type of conditioning	*	70% VP/CY/TBI and 26% CY/TBI	64% TBI-based	92% TBI-based
Acute GVHD	*	II-IV=52%	II-IV=40%	III-IV=28%
Chronic GVHD	*	68%	40%	35% extensive
Disease response	67% CR	87% CR	78% CR	64% CR
TRM	31% at 3 months	35%	40% at 3 years	*
Relapse	*	*	25% at 3 years	13% at 3 years
DFS	50% at 3 years	61% at a median follow up of 26 months	*	44% at 3 years
OS	56% at 3 years	*	55% at 3 years	41% at 3 years
Factors predicting outcome	Phase of disease predicted survival while chemo-sensitivity predicted relapse	*	Prior exposure to FLU is associated with better outcome	CR at HCT and Karnofsky score of 90% predicted better survival

*Not reported

Twenty-six patients with advanced stage and/or refractory CLL have undergone allogeneic stem cell transplantation from HLA-matched related donors at the FHCRC between 1980 and 1999. Patients were a median of 49 (range 14-59) years old. Twenty-one had B-cell CLL, one had T-

CLL and four patients did not undergo phenotyping. One patient had Richter's transformation and one patient evolved to prolymphocytic leukemia prior to transplant. Nineteen patients had received fludarabine with a median of 4 different regimens prior to HSCT. All patients had persistent disease at the time of transplant; 19 were Rai stage IV, 3 were stage III, 2 were stage II, and 2 were Rai stage 0. Patients received conditioning with busulfan (14 mg/kg) and CY (150 mg/kg) or CY/TBI (10-16 Gy). One patient underwent conditioning with etoposide 60 mg/kg, CY 100 mg/kg and 12 Gy TBI. Patients received HSCT from HLA-matched, sibling donors (n=22), syngeneic donors (n=3) or DRB1 mismatched sibling (n=1). Twenty-two patients engrafted. VOD occurred in 12 patients and renal failure occurred in 6 patients. Fourteen patients had GVHD ≥ grade 2 requiring therapy. Thirteen patients (50%) achieved CR by bone marrow morphology, and six of seven evaluable patients achieved CR by flow cytometry. Eighteen patients (69%) have died at a median follow-up of 139 days (range 16-1906 days) from multi-organ failure (n=6), infection (n=5), GVHD + infection (n=2), Relapse (n=2), other/unknown (n=3). Eight patients survive at a median of 39 (range 9.5-101) months. The 5-year actuarial survival is 31% for all patients, and 56% for patients receiving conditioning with TBI after 1992 (n=14).

Five patients with advanced stage and/or refractory CLL (n=4) or CLL transformed to PLL (n=1) were given unrelated HCT between 1990 and 2000 at FHCRC. Median age was 46 (range 35-49) years and median interval between diagnosis and HCT was 22 (range 12-55) months. Patients were conditioned with cyclophosphamide/TBI (13.2Gy). After HCT, three patients had grade II and one grade III acute GVHD. Chronic extensive GVHD developed in 3 patients. Overall 4 patients died, one patient achieved CR, but died from multiorgan failure, one died from complications of a second HCT given for rejection of the first graft, one died from renal failure associated with progressive disease, and one from pneumonia with persistent disease. One patient who was in CR at time of HCT, is alive in CR at 1091 days after HCT.

In summary, conventional allogeneic HCT, while potentially curative as evidenced by the low relapse rates, was associated with a high TRM ranging from 31% to 50% (reviewed in[62]), even in patients less than 50 years of age. Unrelated donor HCT carries even higher risk of TRM with low survival advantage.

E. Reduced-dose regimens for allogeneic HCT

The use of nonmyeloablative regimens are appealing as treatment for patients with CLL for several reasons. First there are data that graft-versus-leukemia (GVL) effects appear to be effective in treating patients with CLL. The reduced toxicity of the nonablative conditioning was anticipated to result in lower TRM as compared to conventional conditioning. Third CLL patients are generally older than usual age where ablative HCT is offered.

The experience with reduced intensity transplants for CLL has been limited. Two large studies using reduced intensity conditioning and HCT for CLL, one from the Cooperative German Transplant Study Group[65] and one from the EBMT representing 29 European centers[66] are summarized in Table.3.

Table 3. Allogeneic reduced-intensity HCT for CLL

	Cooperative German Transplant Study Group (n=30) [65]	EBMT (n=77) [66]
Donor Related Unrelated	 50% 50%	 82% 18%
Age, Median (range), years	50 (12-63)	54 (30-66)
Interval between diagnosis and HCT, median (range), months	48 (12-510)	49 (8-146)
Number of chemotherapy regimens, median (range)	3 (0-8)	3 (0-8)
Disease status at HCT Responsive (CR/PR) Refractory (SD/PD)	 46% 46%	 65% 35%
Fludarabine exposure	33% fludarabine-refractory	82% previous exposure, no report on refractoriness
Type of conditioning	Fludarabine/ busulfan/ rabbit anti-thymocyte globulin	65% low dose TBI or cyclophosphamide-based 35% fludarabine/busulphan or high dose melphalan combinations
Acute GVHD Grade II-IV Grade III-IV	 56% 20%	 34% 16%
Chronic GVHD Limited Extensive	 54% 21%	 58% limited+extensive
Disease response (CR+PR)	(40+53%)	(69%+22%)
TRM All patients Unrelated donor recipients	 15% at 2 years 28% at 2 years	 18% at 1-year
Relapse	*	31% at 2-years
PFS	67%	56% at 2-years
OS	72%	72% at 2-years

*Not reported

SD indicates stable disease; and PD, progressive disease

F. Our preliminary results of nonmyeloablative conditioning and allogeneic HCT for CLL

Based on preclinical studies in a canine model, [67] we developed a nonmyeloablative conditioning regimen for hematopoietic cell transplantation (HCT) for patients with hematological malignancies. The regimen was first used in patients receiving HCT from HLA-

matched related donors[68] and from January 2000, for patients given HCT from HLA-matched unrelated donors. [69,70]

In 2005, we reported our encouraging early results of allogeneic hematopoietic cell transplantation (HCT) after nonmyeloablative conditioning in 64 patients who had advanced chronic lymphocytic leukemia (CLL). Patients were given HCT from related (n = 44) or unrelated (n = 20) donors. Patients had multiple risk factors: median age was 56 years; median interval between diagnosis and HCT was 4.4 years; CCI scores of ≥1 for pretransplantation comorbidities were present in 48% of patients; chemotherapy resistance to pretransplantation salvage treatment was present in 53% of patients and untreated relapse occurred in 11% of patients; there was a median of four prior treatment regimens; and disease refractoriness to at least one regimen was present in all but two patients (97%). Thirty patients (47%) were refractory to one regimen, 23 patients (36%) were refractory to two regimens, and nine patients (14%) were refractory to ≥three regimens. Eighty-eight percent of patients were refractory to fludarabine, 25% of patients were refractory to rituximab, 30% of patients were refractory to alkylating agents, and 22% of patients were refractory to other miscellaneous regimens. In addition, patients had multiple adverse disease burden characteristics: bulky lymphadenopathy (lymph node diameter ≥5 cm, 28%), splenomegaly (47%), CD5/CD9 coexpression of CD38 more than 30% (58%), beta$_2$-microglobulin more than 2.5 µg/mL (53%), ≥50% marrow infiltration with CLL cells (52%), and unfavorable cytogenetics (39%). No significant differences in adverse disease burden characteristics were present between related and unrelated recipients. After HCT, Sixty-one of 64 patients had sustained engraftment, whereas three patients rejected their grafts. The incidences of grades 2, 3, and 4 acute and chronic graft-versus-host disease were 39%, 14%, 2%, and 50%, respectively. Three patients who underwent transplantation in complete remission (CR) remained in CR. The overall response rate among 61 patients with measurable disease was 67% (50% CR), whereas 5% had stable disease. All patients with morphologic CR who were tested by polymerase chain reaction (n = 11) achieved negative molecular results, and one of these patients subsequently experienced disease relapse. The 2-year incidence of relapse/progression was 26%, whereas the 2-year relapse and nonrelapse mortalities were 18% and 22%, respectively. Two-year rates of overall and disease-free survivals were 60% and 52%, respectively. Unrelated HCT resulted in higher CR and lower relapse rates than related HCT. Two-year OS and DFS of related compared with unrelated recipients were 56% *v* 75% (*P* = .33) and 44% *v* 75% (*P* = .15), respectively (Figure 2).

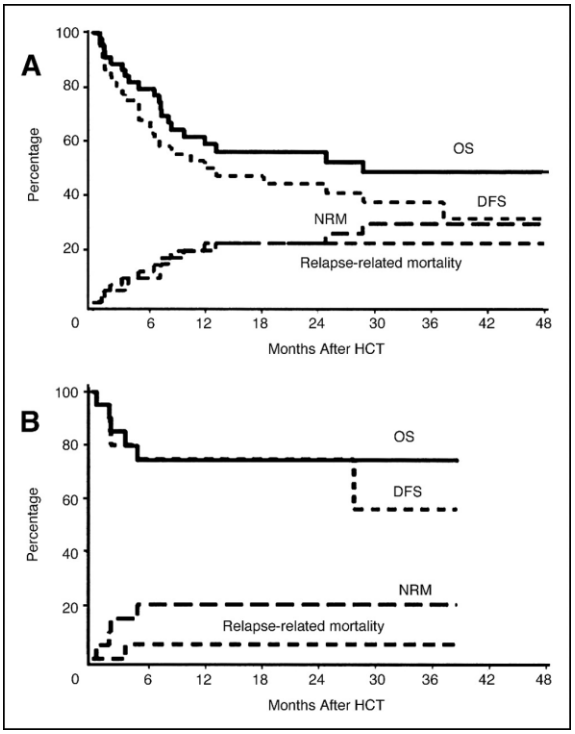


Figure 2. Cumulative incidences of nonrelapse mortality (NRM), relapse-related mortality, overall survival (OS), and disease-free survival (DFS) for (A) related and (B) unrelated recipients.

Recently, Here, we have extended the follow-up to a median of 5 years and have included data on an additional 18 patients. Grafts were from related (n = 52) or unrelated (n = 30) donors. Complete remission (CR) and partial remission were achieved in 55% and 15% of patients, respectively. Higher CR rates were noted after unrelated HCT (67% v 48%). The 5-year incidences of nonrelapse mortality (NRM), progression/relapse, overall survival, and progression-free survival were 23%, 38%, 50%, and 39%, respectively. Among 25 patients initially reported in CR, 8% relapsed and 8% died as a result of NRM, whereas 84% have remained alive and in CR. Among 14 responding patients who were tested and who had molecular eradication of their disease, two died as a result of NRM, two relapsed, and 10 have remained negative. The 5-year prevalence of patients alive after discontinuation of all immunosuppressive medications was 38% (35% for related and 44% for unrelated recipients, Figure 3); the median performance status in each group was 100% and 90%, respectively. Lymphadenopathy > or = 5 cm, but not cytogenetic abnormalities at HCT, predicted relapse. In a risk-stratification model, patients who had lymphadenopathy less than 5 cm and no comorbidities had a 5-year OS of 71%.

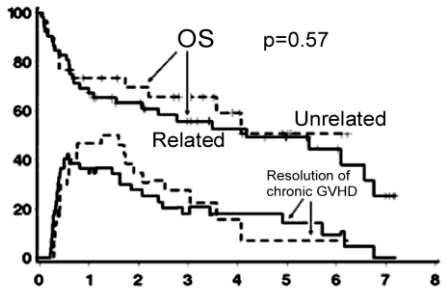


Figure 3. CLL: OS based on donor type and resolution of GVHD following allogeneic HCT.

Outcomes of patients with favorable (normal or 13q deletion) versus unfavorable (all others) cytogenetic abnormalities were comparable (Figure 4). Seven patients had 17p deletion, of whom 4 (57%) are alive and in CR, 1 died with NRM while in CR, and 2 died from relapse.

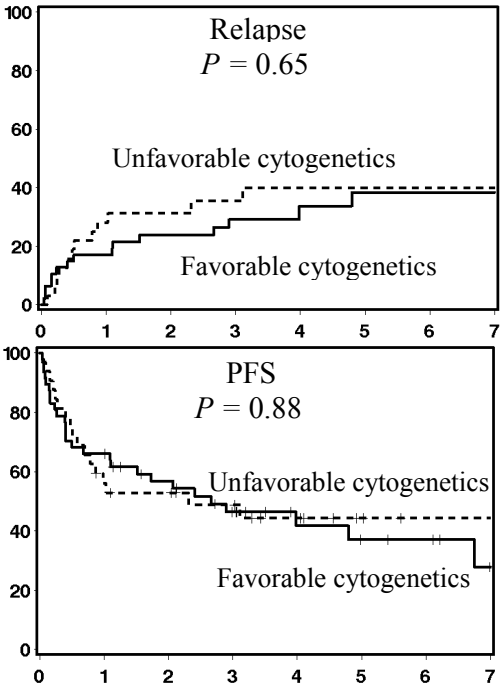


Figure 4. Comparable 5-year relapse and PFS rates among CLL patients with unfavorable (17p deletion, 11q deletion, trisomy 12, and complex) versus favorable (normal and 13q deletion) cytogenetic abnormalities following nonmyeloablative allogeneic HCT.

In summary, Nonmyeloablative HCT resulted in a median survival of 5 years for patients who had fludarabine-refractory CLL with sustained remissions and in the continued resolution of chronic graft-versus-host disease in surviving patients. The approach appeared feasible with acceptable NRM, high rates of disease responses, and a good evidence of GVL effect. Outcomes of related and unrelated recipients were similar, providing the rationale to combine both grafts in this protocol. However, disease relapse remained as a cause of treatment failure with 5-years rate of 26%. NRM was the other cause of treatment failure, 24% at 5-years, and it resulted mainly from GVHD and its complications and infections. We thought to further improve our results by adding peri-transplant Rituximab with the main goal of more robust disease-control and a secondary goal of limiting GVHD and its complications.

G. Update on results of CLL patients with poor prognosis

Recently, investigators from MD Anderson Cancer Center have presented at the American Society of Clinical Oncology 2007 Meeting results of 77 patients with CLL who have failed initial treatment with combination chemo-immunotherapy comprising fludarabine, cyclophosphamide, and rituximab (FCR). Ten of those patients have not required therapy while 67 patients received first salvage therapy with a variety of regimens. Overall response rate was 39% and CR rate was 17%. Median survival after the first salvage therapy was 30 months (28 deaths) and was shorter for patients with PR or refractory disease (10 months). Of interest, 13 patients who failed the first salvage therapy went to receive allogeneic HCT after reduced intensity regimens. Of those 13 patients, 3 died early, one relapsed, 2 in PR, and 7 in CR. Survival at 3 years is 76%. The authors concluded that patients who failed to obtain CR or nodular PR after FCR or first salvage therapy should be considered for allogeneic HCT.[52]

Alternatively, patients with advanced CLL and 17p deletion genomic feature have a very poor prognosis even after intensive chemotherapy. The hierarchical model of Dohner and colleagues identified the prevalence of this genomic aberration to be of 7%.[53] Patients with 17p deletion had only 0.75 year treatment-free interval and 2.7 years median survival from time of diagnosis.[53] Recently, investigators from Germany have shown that patients with 17 p deletion had statistically significantly shorter median PFS (11 versus 24 months, p=0.002) and median OS (15.9 months versus not reached, p<0.001) compared to patients without 17p deletion following fludarabine-based regimens (Figure 5).[54] Similar findings were also reported by investigators of the US Intergroup Phase III Trial E2997, where patients with 17p deletion had median PFS of 0.9 years after initial therapy with fludarabine ± cyclophosphamide.[55] Further, investigators from MD Anderson Cancer Center have shown at the American Society of Hematology 2007 Meeting that 17p deletion +/- other genomic aberrations was the strongest independent predictor for shorter survivals and poor response to the combination FCR as an initial therapy for CLL.[56]

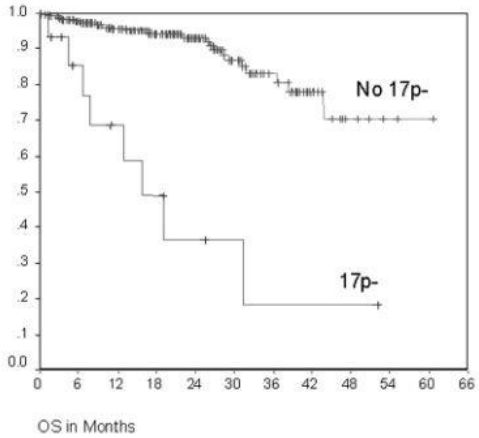


Figure 5. Kaplan-Meier survival for CLL patients (n=375) with versus without 17p deletion following initial treatment with fludarabine ± cyclophosphamide.

Updated results suggest that patients who fail FCR combination chemotherapy at any time point and patients with "de novo" or acquired 17p deletion cytogenetic abnormality, who received induction chemotherapy should be enrolled in nonmyeloablative protocols.

H. Peri-transplant Rituximab to improve early disease control and reduce relapse risk

The cell-surface antigen CD20 is a 297-amino acid transmembrane phosphoprotein that is expressed on more than 90% of mature B-cell leukemias and lymphomas. [71] Rituximab is a chimeric anti-CD20 monoclonal antibody, which has been shown to be active against CLL either as a single agent[72]or in combination with chemotherapy. [73].The proposed mechanisms for the cytotoxic action of rituximab include complement-dependent cytotoxicity,[71]antibody-

dependent cellular cytotoxicity, [71] and signaling-induced apoptosis [74,75] in addition to synergistic effects with chemotherapeutic agents[71] and radiation therapy. [76]

Theory:

The antitumor effects of NM HCT are substantial, but disease progression occurs in a substantial fraction of patients. In our earlier results with nonmyeloablative HCT for patients (n=64) with advanced CLL, we found that the median time to early progression was 3.3 months, while median time to the first sign of disease eradication, complete resolution of cytogenetic abnormalities, by GVL was 3 months. Patients with progressive disease frequently have a larger tumor burden prior to transplant and fail to develop T cell responses to CLL, suggesting that tumor antigens are not efficiently presented or reactive T cells are tolerized by the high tumor burden. Based on these findings, we could hypothesize that improvement of disease control could be achieved by 1) additional treatment in the early few weeks after HCT and 2) earlier and/or stronger generation of GVL effects. Rituximab given days before and after HCT could hypothetically meet these two objectives. Rituximab through antibody-dependent cytotoxicity could prevent disease progression in the early interval after HCT[71]. Further, investigators found that anti-CD20 antibodies, by induction of apoptosis, could promote uptake and cross presentation of cell-derived peptides by antigen-presenting dendritic cells. [77] In these lines, Rituximab could allow cross-priming and generation of specific donor-derived cytotoxic T cells resulting in earlier “switch-on” of GVL effects. [78,79]

Tolerability:

Rituximab, alone or in combination with various chemotherapy regimens, was generally well tolerated in clinical trials in patients with advanced-stage indolent or aggressive B-cell NHL or B-cell CLL. The most common types of adverse events in these trials were infusion-related reactions, hematological adverse events and infections. Infusion-related reactions that occur in the majority of patients, most within 2 hours of the first infusion, are generally mild to moderate flu-like symptoms that usually resolve upon slowing or stopping the infusion, and become less frequent with subsequent infusions. Severe (grade 3/4) reactions, including severe cytokine release syndrome, occur in 10% of patients and may also require supportive care (e.g. analgesic, antihistamine, oxygen, intravenous fluids, bronchodilators, vasopressors and/or corticosteroids). The true incidence of rituximab-related infections has not been determined, but nonrandomized trial data showed a 31% overall (19% bacterial, 10% viral) and 2–4% incidence of severe infectious events. (Biogen Idec Inc., Genentech Inc. Rituxan® (Rituximab): prescribing information. 2006 Feb 28; and Roche Registration Limited. MabThera 100mg (concentrate for solution for infusion): summary of product characteristics[online]. Available from URL: www.rocheuk.com [Accessed 2006 Mar 10]). Overall, treatment with rituximab, in combination with fludarabine or as part of the FCR regimen, was well tolerated by patients with B-cell CLL in phase II trials[80-82], Incidences of severe neutropenia and infections were comparable to those seen with fludarabine-based regimens[81]. Myelosuppression was considered predominantly chemotherapy, rather than rituximab related. [80-82]

Pharmacokinetics:

Some facts are known from the intravenous administration of Rituximab in patients with B-cell malignancies:

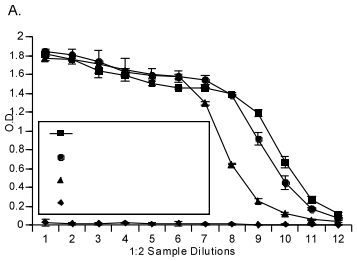


Fig. 6 Rituximab ELISA. The mean of triplicate assays was 41.1 +/- 5.5 μg/ml and 11.8 +/- 0.7 μg/ml for 2 spiked samples of 40.0 and 10.0 μg/ml.

1) The drug serum concentration is directly proportional to the administered dose and to the clinical response [71]. However, they are inversely correlated to the absolute level of circulating peripheral B cells and the tumor bulk measurements at baseline. [83] 2) Repeated administration results in accumulation of the drug due to reduction in the population of both normal and malignant CD20-positive B cells and the reduction or saturation of CD20-binding sites[71]. 3) There is wide interindividual variability reflecting the variable tumor responsiveness and burden among patients with B-cell malignancies. 4) Clinically relevant drug concentrations were detectable by ELISA in the serum of these patients up to 6 months after treatment [71]. 5) Rituximab serum concentrations were significantly lower in patients with B-cell CLL. [84] This is probably due to the presence of the soluble CD20 antigens, found in patients with CLL, that act as a 'sink' for rituximab, increasing clearance and reducing delivery of the drug to malignant B cells.

Very limited information is available though on pharmacokinetics of Rituximab in the settings of allogeneic HCT. We aim to study these pharmacokinetics in the current patient population and ask questions about serum concentrations and correlations to drug dose and clinical responses at different time points after HCT. There is no commercial assay available to determine serum concentrations of rituximab. The Maloney lab has developed a murine anti-idiotype (anti-Id) monoclonal antibody (18C9) which binds specifically to rituximab, to the murine parent antibody used to construct rituximab, and to Fab' fragments of these antibodies and which does not cross-react with other human IgG1 antibodies or human serum (Fig. 6). The 18C9 anti-Id was used to develop an ELISA to measure serum rituximab concentrations. This assay can quantify rituximab levels in fresh, refrigerated or frozen human serum to <1 μg/ml. We will use this assay to measure rituximab levels in patients before and after allogeneic HCT; we will correlate serum levels with response and relapse rates, and evaluate the effectiveness of our dosing regimen in consistently achieving trough serum rituximab levels of >25 μg/mL based on a reported correlation of trough levels >25 μg/mL with disease response [83].

Genetic determinants of responsiveness to Rituximab

Polymorphisms of the FcγRIIIa receptor predominantly expressed on NK cells, have been shown to affect the antitumor activity of Rituximab in some but not all studies. [85-87] The Fc γRIIIa of individuals that are homozygous for valine at position 158 (158V/V) has a higher affinity for human IgG1 than FcγRIIIa that are homozygous for F (158F/F), and confers greater antibody dependent cell mediated cytotoxicity in vitro. [85] Patients with 158V/V have better and more durable responses to Rituximab than patients with 158F/F, while 158 V/F heterozygotes have a variable response. Maloney lab has developed a method to genotype FC γRIIIa by PCR and

single-strand conformation polymorphism (SSCP). A region of exon 4 of FC γRIIIa was amplified by PCR and the 125 bp product verified on a 2% agarose gel and by sequencing (**Fig 7A**). The PCR products were denatured and then electrophoresed through pre-cast 20% TBE in Xcell II Mini-Cell gel electrophoresis unit in 1% TBE buffer to separate the different strands (**Fig. 7**). This is a sensitive method to identify Fc γRIII polymorphisms from small amounts of DNA isolated from patient or donor cells. A similar method has been developed to detect the position 131 (histidine/arginine) polymorphism in the Fc receptor CD32, which has also been correlated with Rituximab response in some studies. [85] This method will be used to determine the genotype of these polymorphisms in hematopoietic cells pre and post NM HCT.

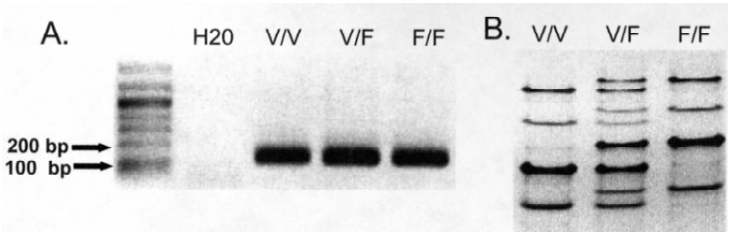


Figure 7: (A) PCR amplification of FCγRIIIa exon 4 from 3 patients, (B) SSCP analysis of the 3 samples, V/V, V/F, and F/F. Data from D. Maloney.

Patients with FcR polymorphisms that provide high affinity binding for human IgG1 had higher response rates or longer duration of remission following Rituximab therapy in some studies. This has not been evaluated in the context of allogeneic HCT, where the FcR status may change pre and posttransplant. We hypothesize that CD16 158 (VV) and CD32 131(HH) will be associated with increased efficacy and better outcome. It is unknown whether donor or recipient Fc γRIII genotype will be more relevant for the efficacy of Rituximab in NM HCT.

GVHD

Cumulative incidence of extensive chronic GVHD in our 82 CLL patients was 50%. We will study in this protocol whether the addition of Rituximab would reduce chronic GVHD rates in comparison to our historical CLL patients given nonmyeloablative HCT. This is based on several reports indicating efficacy of Rituximab in treating refractory chronic GVHD. [88-92] Responses have been limited to cutaneous and musculoskeletal features[88-90] with few exceptions. [93] The precise role of B cells in chronic GVHD and the mechanism of benefit from rituximab remain controversial. Autoantibodies have been implicated in the pathogenesis of chronic GVHD by some groups [94], and rituximab may function by suppressing their production [89]. Alternately, rituximab-mediated B-cell depletion may modulate T-cell alloreactivity, as in other autoimmune diseases [95]. Given the clinical benefit in established chronic GVHD, administration of rituximab in the early post-transplant period may have a prophylactic effect, reducing the incidence or severity of chronic GVHD.

I. Assessment of Pretransplant Comorbidities

Charlson Comorbidity Index (CCI) is a well-known simple index to score comorbidities which was developed to provide prediction of risks of survival after treatment of chronic medical illnesses.[96] The Seattle team used this index in 2004 to score pretransplant comorbidities among patients diagnosed with hematological malignancies and offered HCT. The CCI was helpful in predicting risks of non-relapse mortality and survival[97,98]. However, the CCI showed a limited ability in capturing comorbidities among the transplanted population. Therefore, the same authors investigated the possibility of modifying the original CCI to better

capture comorbidities among transplanted patients.[99] They tried to a) better define previously identified comorbidities utilizing pretransplant laboratory data, b) investigate additional HCT-related comorbidities, and c) establish comorbidity scores that were suited for HCT. This resulted in developing a new HCT-specific comorbidity index (HCT-CI), which captured comorbidities among 62% of patients with scores >0 compared to 12% captured by the original CCI.[99] Additionally, the new index was superior to the old CCI in prediction of survival (likelihood ratio of 23.7 versus 7.1 and *c* statistics of 0.661 versus 0.561, *P*=<0.0001, respectively). Recently, the HCT-CI was shown to be an important prognostic and risk-assessment factor in comparing outcomes of patients diagnosed with CLL and given myeloablative versus nonmyeloablative HCT. [100]

3. Proposal

We intend to study whether allogeneic HCT after nonmyeloablative conditioning and peri-HCT Rituximab is effective therapy for CLL. We intend to demonstrate this by comparing survival of patients transplanted under this protocol to that achieved in the historical controls after treatment with CAMPATH.

4. Objectives

A. Primary objective
   1. Determine whether nonmyeloablative conditioning and allogeneic HCT improves survival at 18 months for patients with fludarabine-refractory, FCR-failed, or del 17p CLL over that of historical controls (45% at 18 months) given CAMPATH-1H.[14]

B. Secondary objectives
   1. Estimate the overall response rate (CR + PR) by standard morphologic, flow cytometric, and molecular techniques.

   2. Assess the rate of relapse/progression.

   3. Define incidences of RRT and infections within the first 100 days and the incidence of TRM within the first year.

   4. Estimate incidences of grade II-III and III-IV acute GVHD and chronic GVHD.

   5. Assess the impacts of Rituximab

      a. Determine whether the addition of Rituximab to the nonmyeloablative conditioning and allogeneic HCT improves survival at 18 months over our historical data (57% at 18 months).

b. Determine the incidence of serious adverse events with the addition of Rituximab in comparison to historical data of unrelated nonmyeloablative HCT.
c. Evaluate the pharmacokinetics of Rituximab.
d. Evaluate B-cell and T-cell immune reconstitution in comparison to historical data of unrelated nonmyeloablative HCT.
e. Describe donor and host polymorphisms of the FCγRIIIa receptor and CD32 and evaluate their impact on disease response and relapse.

6. Graft-versus-leukemia analysis

a. Investigating mechanism of disease resistance in relapsed/non-responding patients
b. Isolation of donor cytotoxic T lymphocytes specific for host minor histocompatibility antigens

5. Patient Selection

A. Inclusions:

1. Patients with a diagnosis of CLL (or small lymphocytic lymphoma) or Diagnosis of CLL that progresses to prolymphocytic leukemia (PLL).
2. Patients with B-Cell CLL or PLL who:

   a. Failed to meet NCI Working Group criteria[2] (Appendix H) for complete or partial response after 2 cycles of therapy with a regimen containing fludarabine (or another nucleoside analog, e.g. 2-CDA, pentostatin) or with disease relapse within 12 months after completing therapy with a fludarabine (or another nucleoside analog) containing regimen.

   b. Failed FCR or PCR combination chemotherapy at any time point.

   c. Patients with novo or acquired "17p deletion" cytogenetic abnormality. Patients should have received induction treatment but could be transplanted in 1st CR.
3. Patients who have suitable HLA-matched related or unrelated donors willing to receive G-CSF, undergo leukapheresis to collect PBMC, and to donate stem cells.
4. Patients who are older than 18 years old.

B. Exclusions:

1. Infection with HIV.
2. Active diagnosis of CNS involvement with CLL. For LP requirement, see Appendix N.
3. Patients unwilling to use contraceptive techniques before and for 12 months after HCT
4. Pregnant women or females who are breastfeeding.
5. The addition of cytotoxic agents for "cytoreduction" with the exception of tyrosine kinase inhibitors (such as imatinib mesylate), cytokine therapy, hydroxyurea, low dose cytarabine, chlorambucil, or rituxan will not be allowed within three weeks of the initiation of conditioning.

6. Active bacterial or fungal infections unresponsive to medical therapy.
7. Performance status:
    a. Karnofsky score < 60 (see **Appendix B**) for adult patients
8. Severe organ dysfunction:
    a. Cardiovascular:
        i. Cardiac ejection fraction < 40%. Ejection fraction is required if age > 50 years or there is a history of prior transplant, anthracycline exposure or history of cardiac disease.
        ii. Poorly controlled hypertension despite multiple antihypertensives.

    b. Pulmonary:
        i. DLCO < 40%, TLC <40%, FEV1 <40% and/or requiring continuous supplementary oxygen, or severe deficits in pulmonary function testing as defined by pulmonary consultant service.
        ii. The FHCRC PI of the study must approve of enrollment of all patients with pulmonary nodules.

    c. Hepatic:
    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, hepatic damage with bridging fibrosis, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3 mg/dl, or symptomatic biliary disease.

9. Patients with active non-hematologic malignancies (except non-melanoma skin cancers). This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy.
10. Patients with a history of non-hematologic malignancies (except non-melanoma skin cancers) currently in a complete remission, who are less than 5 years from the time of complete remission, and have a >20% risk of disease recurrence.
    This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy.

6. Donor Selection

A. Inclusions

1. Related donors

When more than one potential donor exists, priority should be given to donors based on HLA identity > CMV seronegativity > ABO compatibility > sex matching. Eligibility guidelines for donor PBMC apheresis based on immunization status are shown in Appendix A.

a. Donor who is HLA phenotypically or genotypically identical at the allele level at HLA-A, -B, -C, -DRB1, and -DQB1.

b. Donor must consent to G-CSF administration and leukapheresis.

c. Donor must have adequate veins for leukapheresis or agree to placement of central venous catheter (femoral, subclavian).

d. Only G-CSF mobilized PBMC only will be permitted as a HSC source on this protocol.

2. **Unrelated donors**

a. 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**).

b. 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.

c. 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.

d. Only G-CSF mobilized PBMC will be permitted as a HSC source on this protocol.

B. Exclusions

1. Age < 12 years
2. Identical twin
3. Pregnancy
4. Infection with HIV
5. Inability to achieve adequate venous access
6. Known allergy to filgrastim (G-CSF)
7. Current serious systemic illness

7. Informed Consent

Patient

A conference conducted by the outpatient, attending physician will be held with the patient and family to discuss this study and alternative treatments available for treatment of advanced CLL. The goals of the study, requirement for data collection, and requirement for release of medical records will be discussed with the patient. All potential risks associated with the use of fludarabine, low dose TBI, Rituximab, immunosuppressive drugs, HCT, and disease progression/recurrence will be discussed as objectively as possible. Discussion of potential complications should include graft rejection, GVHD, infections, and death. It should be explained that patients offered this protocol have advanced malignancy with life expectancy of about 10 months with conventional treatments, and are at high risk of early transplant mortality with conventional allogeneic HCT and high relapse rate after an autologous transplant. It should also be explained to the patient that our preliminary results showed no difference in outcomes after related or unrelated donor nonmyeloablative allogeneic HCT.

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. The patient is also required to sign a HIPPA consent form.

Donor

For the family member stem cell donor, the procedure for collecting peripheral blood mononuclear cells and toxicities of G-CSF will be explained. The donor should be counseled as to the risks of treatment with G-CSF and be informed that leukapheresis at several time points may be necessary. Informed consent from the patient and donor will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. All patients enrolled at collaborating centers will engage in institution-specific informed consent conferences after completion of the pre-transplant evaluation. Informed consent from the donor and patient will be obtained using a form approved by the Institutional Review Board for each treatment center.

Informed consent from the unrelated donor will be obtained according to NMDP regulations.

8. Protocol Registration

A. FHCRC patients: Eligible patients will be assigned to the protocol by the Clinical Coordinator who will register the patient 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.

B. Collaborating institutions: Eligible patients will be identified by the principal investigator of the collaborating institution who will register the patient with the FHCRC Registration Office. Registration will include completion of the eligibility checklist and demographic form (Appendix L). This form and a copy of the signed informed consent will be faxed to the Trial Coordinator (206-667-5378). Questions regarding eligibility or protocol information should be directed to the Principal Investigators, Mohamed Sorror, M.D. (206-667-2765) or David Maloney, M.D. (206-667-5616).

9. Plan of Treatment

A. Outline of treatment plan

The plan for treatment is shown below in Figure 8 and described in Table 4.

B. HCT

Patients will undergo HCT as soon as eligibility requirements are met and donor leukapheresis, for related recipients, is scheduled (in practice approximately 7-21 days after the arrival conference). For unrelated recipients, HC source will be G-PBMC collected as per NMDP or other regulatory protocol. Two (12 liters) leukaphereses will be obtained on consecutive days, and collections will be infused together on day "0".

Patients will only be admitted as medically necessary for control of transplant complications or for the infusion of HCT.

For unrelated recipients, standard cryopreservation of 10% of the G-PBMC will take place for DLI.

C. Cytoreduction

Cytoreduction and /or radiation therapy may be given by the referring physician or the attending physician as determined on clinical grounds or to meet eligibility requirements of the protocol for patients with advanced malignancy or to reduce tumor bulk. Cytoreduction can be performed with any appropriate therapy for CLL. The choice of therapy will depend on prior regimens and the current disease status and may be selected at the discretion of the attending physician and/or the referring physician. However, no intensive chemotherapy can be given within three weeks (or the interval in which a cycle of standard chemotherapy would be administered in a non-transplant setting) prior to initiating the nonmyeloablative transplant conditioning (see exclusion criteria page 19). The need for this therapy should be discussed with the principal investigator. The referring oncologist may be asked to administer this therapy. Alemtuzumab should be given only >60 days before allogeneic HCT to avoid its lingering immunosuppressive effect on the donor graft.

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 cytokine therapy, low dose cytarabine, chlorambucil, or rituxan.

D. Conditioning regimen

Conditioning will begin four days prior to stem cell infusion.

i. On days –4 to –2 patients will receive fludarabine intravenously at a dose of 30 mg/m^2/day.

ii. On day -3, patients will receive rituximab intravenously at a dose of 375 mg/m^2.
- Follow SCCA general oncology guidelines for infusion of drug
- Follow SCCA general oncology guidelines for rituximab pre-meds and prn reactions

iii. On day 0, 200cGy of TBI will be administered at 6-10 cGy/min from a linear accelerator. TBI will be administered between 10:00 a.m. and 2:00 p.m. to avoid proximity to the start of immunosuppression.

iv. G-PBMC from an HLA-matched donor, will be infused on day 0, following TBI.

v. Patients will receive rituximab intravenously at a dose of 375 mg/m^2 at three time points after stem cell transplantation. These time points are set up at days 10+, 24+, and 38+. However, if there are clinical reasons that prohibit giving Rituximab at one of these specified time points the dose can be delayed to a maximum of 5 days. Following doses should be attempted to be given at the scheduled time points. If doses need to be delayed more than 5 days from the specified time points, then patient should only get the next scheduled dose Day 38+ dose can be delayed up to 100 days after HCT.

Table 4. Conditioning Schema and Immunosuppression Schedule

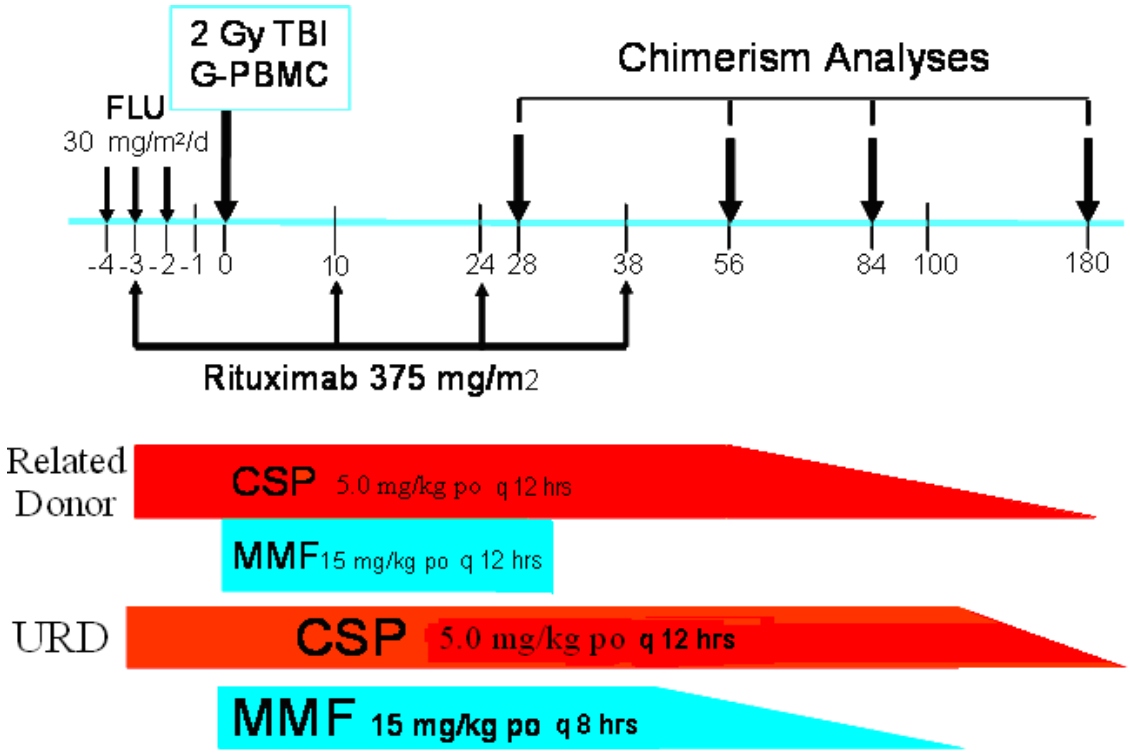
Day number	-4	-3	-2	-1	0	+1	+10	+24	+27	+38	+40	+56	+96	+100	+180
Fludarabine	X	X	X												
TBI					200 cGy										
Rituximab		X					X[A]	X[A]		X[A]					
Stem cell infusion					Infusion										
Related recipients															
CSP		Start	→	→	→	→	→	→		→	→	Taper[B]	→	→	Stop
MMF					Start[C]	→	→	→	Stop						
[A]These are recommended time points that can be delayed for a maximum of 5 days for clinical reasons. [B]Taper will be a ~6% dose reduction per week x 17 weeks. [C]The first dose of MMF is to be given 4-6 hours after the stem cell infusion.															
Unrelated recipients															
CSP		Start	→	→	→	→	→	→		→	→	→	→	Taper[C]	Stop
MMF					Start[D]	→	→	→	→	→	Taper[D]	→	Stop		
[C]Taper will be a ~8% dose reduction per week x 11 weeks. [D]The first dose of MMF is to be given 4-6 hours after the stem cell infusion															

E. Immunosuppression

- Postgrafting immunosuppression with CSP and MMF will be used to permit engraftment and provide GVHD prophylaxis.
- Day –3. Commence CSP at 5.0 mg/kg PO Q12 hrs, continue to day +56 *for related* and day +100 *for unrelated recipients*, then taper to day +180. CSP should be routinely taken at 9:00 a.m. and 9:00 p.m.
- Day 0: **After** HCT on day 0, MMF will be given based on adjusted body weight, at 15 mg/kg PO, 4-6 hours after HCT is complete.
    - For *related recipients* MMF to be given at 15 mg/kg PO Q12 hrs, continue to day +27, then stop abruptly.
    - For *unrelated recipients* MMF to be given at 15 mg/kg PO Q8 hrs, continue to day +40, then start to taper to day +96

CSP and MMF treatment and taper schedules are described below and depicted in Table 4.

Figure 8. Diagram of treatment plan



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)

1. CSP
    a. *CSP Administration Plan*
        i. Starting dose: CSP (Neoral is preferred) is given orally based on adjusted body weight at 5.0 mg/kg Q12 hrs PO from starting day –3. Doses should be adjusted to maintain high therapeutic CSP levels as discussed below.
        ii. If there is no evidence of GVHD, ***for related recipients:*** a CSP taper will be initiated on day +56. CSP will be tapered so that patients will be off of CSP by day +180 (approximately 6% decrease every seven days). ***For unrelated recipients:*** a CSP is to be tapered from day +100 at 8% of the pre-taper therapeutic dose per week to be discontinued at the end of 11

weeks (day +180). The referring physician, who will receive explicit instructions and guidelines for detecting and managing GVHD, will manage this.

iii. If there is evidence of chronic GVHD, then the standard recommendations for chronic GVHD with CSP/prednisone will be followed.

iv. If there is evidence of CLL disease progression, please see section K.

b. 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 A) during the 28 days.

Table A: **CSP Dose Adjustment**

	CSP Level to Target Using LC-MS/MS Method	**CSP Level to Target Using Immunoassay Method**
Day "0"- Day +28 Whole blood "trough" (11-12 hrs from prior dose)	400 ng/ml	500 ng/ml (upper end therapeutic range for this method)
After Day +28	120 - 360 ng/ml	150 - 450 ng/ml
Levels >480 mg/ml by LC-MS/MS Method • **with or without CSP toxicity** • **decrease GFR ≥50%** • **increase creatinine 2x baseline due to CSP**	25% dose reduction	N/A
Levels >600 mg/ml by Immunoassay Method • **with or without CSP toxicity** • **Decrease GFR ≥50%** • **increase creatinine 2x baseline due to CSP**	N/A	25% dose reduction
Patients on Hemodialysis	320 ng/ml	400 ng/ml

iii. CSP Monitoring: CSP determinations should be performed on a twice weekly basis for the first month and then weekly until day +56 ***for related*** and day +100 ***for unrelated recipients*** unless high levels are detected (i.e., >600ng/ml), or toxicity is suspected in which case more frequent monitoring will be performed as clinically indicated. Routine monitoring of CSP will not be required for patients on a CSP taper after day + 140 unless clinically indicated.

iv. CSP Dose Adjustment: Initial high Cyclosporine (CSP) doses are required based on the pre clinical nonmyeloablative canine studies, which used an equivalent dose to establish an allograft. After day +28, CSP levels typical for related or unrelated HCT will be targeted. Dose reduction should only be made if CSP toxicity is present, and/or levels exceed values provided in Table A. There are two methods for calculating CSP levels. Table A 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.

vi. Dose reductions should only be made if CSP toxicity is present or levels exceed 480 ng/ml - 600ng/ml depending on method (see Table A), 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

Oral Sandimmune may be substituted for oral Neoral.

viii. Patients requiring hemodialysis should be have CSP levels maintained in the high therapeutic range (Table A).

ix. Drugs that may affect CSP levels are:

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		
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.*

2. MMF

a. Initiating MMF therapy: ***For related recipients:*** Oral administration of MMF will be at a daily total dose of 30 mg/kg/day based on adjusted body weight (15 mg/kg/d every 12)

For unrelated recipients: Oral administration of MMF will be at 15 mg/kg Q8 hours (45mg/kg/day), based on adjusted body weight.

b. The first dose will be on the evening of day 0 (i.e. first dose to follow 4-6 hours after stem cell infusion).

c. Doses will be rounded to the nearest 250 mg (capsules are 250 mg).

d. If there is nausea and vomiting at any time preventing the oral administration of MMF, MMF should be administered intravenously at 15 mg/kg Q8 hours.

e. Stoppage/Tapering of MMF: ***For related recipients,*** MMF will be given until day +27 post-transplant and then stopped without tapering in the absence of GVHD that requires therapy (e.g. steroids). If treatment for GVHD is required prior to day +27, MMF will be continued at full dose until a steroid taper begins.

For unrelated recipients, MMF will be given based on adjusted body weight daily at 15 mg/kg Q8 hrs until day +40 post transplant and then tapered by 12%/week to be discontinued after day +96, in the absence of GVHD requiring therapy.

f. Guidelines for MMF dose adjustment for drug toxicity: If in the clinical judgment of the investigator and/or attending physician the observed toxicity is related to MMF administration, a dose adjustment will 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). Based on previous organ transplant studies, dose adjustments are likely to occur because of hematopoietic or rarely gastro-intestinal adverse effects.

i. Neutropenia: A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim). G-CSF may be started at 5μg/kg subcutaneously qd to facilitate neutrophil recovery for patients with ANC <750/ul 21 days after HCT. Dose adjustments of MMF will only be made for severe neutropenia that develops or persists after day 21 post-transplant (ANC < 100/μl for > 5 days) that is refractory to G-CSF treatment independent of any other toxicity. In this case dose reductions could be made and should be conservative (20-25%). MMF should only be discontinued temporarily. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides. Any planned dose adjustments for hematologic toxicity **must** be discussed with the principal investigator.

ii. Gastrointestinal toxicity: In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea and is considered to be due to MMF, a 20% dose reduction will occur first and if there is no

improvement, MMF will be reduced a further 20%. For severe G.I. toxicity related to MMF, then MMF may be stopped. Patients should be evaluated by a Gastroenterology consultant to determine the need for dose adjustments for this indication.

F. Collection and infusion of donor G-PBMC

1. G-CSF Administration to Donors:

For related recipients: From day -4 to day –0, all PBSC donors will receive G-CSF, at a dose of 16 μg/kg/day, for 5 consecutive days (Table 5). G-CSF will be administered by a subcutaneous daily injection. The schedule of G-CSF administration and PBSC collections can only be ascertained once day 0 is identified. Once a treatment regimen schedule has been fixed and the schedule of G-CSF administration and PBSC collections made this has to be confirmed with the personnel in the apheresis room. Day 0 should be fixed on a Tuesday-Thursday.

For unrelated recipients: Timing of G-PBMC 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 5) 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.

Table 5. Treatment Schema for donor

Day	**-5**	**-4**	**-3**	**-2**	**-1**	**0**
G-CSF (10-16 ug/kg)	X	X	X	X	X	
G-PBMC collection					X	X

2. G-PBMC Collection:

For related recipients: donors will preferably undergo vein-to-vein collections or may receive an appropriate catheter inserted on or before day of apheresis. Two 12-liter leukaphereses on consecutive days will be obtained, and cells will be infused together on day 0. First, PBSCs will be collected in the afternoon of day -1, stored in the refrigerator at 4ºC overnight. A second collection will be performed the following afternoon and both collections will be transfused on day 0. If $< 5 \times 10^6$ CD34+ cells/kg are collected an additional day of collection will be performed. If PBSCs cannot be collected by a vein-to-vein technique, a percutaneous Mahurkar catheter will be inserted. General procedures will include the use of a standard apheresis machine (COBE Spectra, Lakewood Colo.), and processing up to 16 liters of whole blood during the collection

For unrelated recipients: G-PBMC scheduling and collection is arranged through the NMDP. The schedule of GCSF administration and collection of PBMC is

determined as per NMDP protocol. The physician responsible for HC collection will obtain informed consent from the donor.

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

G. ABO incompatibility

All patients with ABO incompatibility should be evaluated and treated according to the standard practice of the individual institution. Recommendations are provided in Appendix C. It should be noted that two cases of recipient hemolysis have been documented in patients with minor ABO mismatch with their donor. The suspected cause is donor anti-host hemagglutinin production from "passenger lymphocytes" in the donor G-PBMC that may expand posttransplant[101]. Therefore, these patients should be monitored and treated aggressively when there is any evidence of hemolysis.

H. Post-transplant growth factors

Patients are not eligible to receive post-transplant growth factors such as G-CSF during the first 21 days. Growth factors should not be given unless severe persistent neutropenia develops or persists past day 21 post-transplant (ANC <500/μl).

I. Infection prophylaxis

Patients should receive prophylaxis for PCP, VZV, HSV, CMV and candida according to the standard practice of the individual institution for a conventional allogeneic HCT. Recommended infectious prophylaxis is provided in Appendix D. Standard CMV monitoring and prophylaxis should commence at the time of initial transplant and should continue until 1 year post HSCT. Recommendations for monitoring and evaluation of infectious complications are specified in Appendix D. In case of low donor chimerism or disease progression that necessitates transfer of DLI to the patient, modifications in infection prophylaxis will be made according to that protocol.

J. Chimerism

1. Definition

For the purposes of this protocol, definitions will be:

a. M*ixed 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.
b. *Full donor chimerism* is defined as > 95% donor CD3+ T cells. Mixed or full donor chimerism will be evidence of *donor engraftment*

c. *Increasing donor chimerism* is defined as an absolute increase of 20% of donor CD3+ T cells over the previous chimerism evaluation.
d. *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.
e. *Decreasing donor chimerism* is defined as an absolute decrease of ≥ 20% of CD3+ T cell chimerism over the previous month.

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 nuclear cell fraction from marrow aspirates.

2. Evaluation

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 (CD56) compartments. (See Patient Post Transplant Evaluation section for instructions and exceptions).

3. Continuation of immunosuppression

a. In case of low or decreasing donor chimerism. Immunosuppression should be continued or reinitiated at full dose until the low donor chimerism is corrected or the patient may be eligible for another protocol for DLI according to institutional practice.
b. Patients who reject their graft may be eligible for a second allogeneic transplant on other protocols.

4. **Definition of mixed donor/host chimerism, engraftment, graft failure and rejection**. For the purposes of this protocol, *mixed chimerism* will be defined as the detection of donor T cells (CD3+) and granulocytes (CD 33+), as a proportion of the total T cell and granulocyte population, respectively, of greater than 5% and less than 95% in the peripheral blood. *Full donor chimerism* is defined as > 95% donor CD3+ T cells. Mixed or full donor chimerism will be evidence of *donor engraftment. Increasing donor chimerism* is defined as an absolute increase of 20% of CD3+ T cells over the previous chimerism evaluation. . *Decreasing donor chimerism* is defined as an absolute decrease of 20% of CD3+ T cell chimerism over the previous month. *Low donor chimerism* is defined as < 40% CD3+ T cells after HCT. Low donor chimerism

should always be confirmed with repeat peripheral blood T cell and granulocyte chimerism analysis. A DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or FISH studies or VNTR) of the patient and donor will be used to quantitate chimerism of sorted peripheral blood T-cells (CD3+) and granulocytes (CD 33+). The same assay should be used in a given patient for repeated studies of chimerism. This DNA based analysis will also be performed on the whole nucleated cell fraction from marrow aspirates. Therapeutic decisions (i.e. DLI) will be made based on the results of sorted T-cell studies of *peripheral blood*. For the purposes of this protocol, *rejection* is defined as the inability to detect or loss of detection of greater than 5% donor T cells (CD3+) as a proportion of the total T cell population, respectively, after nonmyeloablative HCT. Also for the purposes of this protocol, *graft failure* is defined as grade IV thrombocytopenia and/or neutropenia after day 21 that continue, uninterrupted, for >3 weeks and is refractory to transfusions and growth factor support, respectively.

K. Management of disease progression

In case of disease progression and >40% T-cell donor chimerism. Evidence of substantial disease progression (as defined in Appendix H) will be an indication for therapeutic intervention. In part, this will be dependent on where a patient is relative to the standard tapering schedule. If the attending physician believes that the patient requires very aggressive therapy, the case will be presented to the institutions' patient review committee. Otherwise, priority should be given to rapid reduction of immunosuppression, option (i) below. Therapeutic options include:

1. Early discontinuation of immunosuppression (prior to day +28 for related and day +100 for unrelated recipients). This should be considered the first therapeutic maneuver. If there is no GVHD, MMF is to be stopped if still being taken, and CSP tapered over 2 weeks. Bone marrow aspirate and blood chimerism studies will be performed when off immunosuppression after 2 weeks. If disease continues to progress 2 weeks after stoppage of all immunosuppression, <20% increase in donor chimerism and there is no GVHD, patients will be considered as treatment failure. The patient may then be eligible for DLI treatment according to institutional practice.

2. Early discontinuation of immunosuppression (between days +28 and +180 for related and days +100 and +180 for unrelated recipients). If there is no

GVHD, CSP is to be stopped. Bone marrow aspirate and blood chimerism studies will be performed when off immunosuppression after 4 weeks. If disease continues to progress 2 weeks after stoppage of all immunosuppression, T cell chimerism has not significantly increased (<20% increase) and there is no GVHD, patients will be considered as treatment failure. The patient may then be eligible for DLI treatment or according to institutional practice.

3. 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, the patient may then be eligible for DLI treatment or according to institutional practice.

4. Conventional allogeneic HCT. This option should be discussed with the institutions' patient review committee and the principal investigator. Patients who undergo conventional allogeneic HCT will be removed from the protocol at that time.

10. Patient and Donor Evaluations

A. Patient Pretransplant Baseline Evaluation

1. History: A complete history with full details of the patient's prior treatment and response

2. Assessment of patient pretransplant comorbidities and scoring them using the HCT-Comorbidity Index (HCT-CI; see Appendix Q).

3. Careful physical exam with determination of Karnofsky score (Appendix B).

4. Complete blood count (CBC), serum sodium, potassium, chloride, CO_2, creatinine, BUN, uric acid, calcium, magnesium, phosphate, total bilirubin, alkaline phosphatase, AST, ALT, ABO/Rh typing, hepatitis screen, CMV, VZV, HSV and toxoplasma serology, and anti-HIV serology.

5. Chest X-ray (CXR), PA and lateral views.

6. Pulmonary function tests with corrected DLCO.

7. MUGA scan or echocardiogram to evaluate cardiac ejection fraction for patients > 50 years of age, or history of cardiac disease or prior transplant or anthracycline exposure.

Additionally, see Table 6 for further pre-transplant evaluations.

Table 6: Disease-Specific Pre-Transplant Evaluations

Note: Bone marrow aspirates and biopsies are recommended to be **bilateral** and to be collected within **30 days** of treatment. See Tables 7, 8 and 9 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	
Bone marrow biopsy		
Pathology	Clinical	
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	**for patients not in CR*
Rituxan trough levels – **see comment*	Research - Maloney Lab	**Should be done immediately prior to the Day -3 dose*
Imaging		
CT of chest, abdomen, pelvis (neck if indicated)	Clinical	

Pre-conditioning/Pre-transplant Research Studies **only** for FHCRC patients who have signed **Consent D;**

1. Send 40cc heparinized blood and one 10ml red top tube to the Maloney Lab (206-667-4284; FHCRC D1-331) for cryopreservation of tumor cells. Label the samples "Protocol 1840." These cells will be used as a possible source of leukemia against which the efficacy of killer T cells could be evaluated. The option of leukapheresis for sample acquisition will be discussed with the patient and Attending Physician.

2. Leukapheresis may be done in order to collect a large volume of mononuclear cells. Leukapheresis will be performed using the apheresis machines at the Seattle Cancer Care Alliance and will be done prior to the start of transplant conditioning.

B. Patient Post-transplant Evaluation

1. See Table 7 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. CBC three times a week, or more often if clinically indicated, from day 0 until day +28, and twice weekly until 2 months post-transplant or later if clinically indicated.

3. Electrolyte panel and renal and hepatic function 3 times per week until day +28 and then weekly.

4. Evaluate at Day +84

   GVHD evaluation guidelines:

   a. History and physical exam with attention to the evaluation of chronic GVHD (Appendix G)
   b. Complete blood count, serum IgG, and serum total bilirubin, alkaline phosphatase, ALT, and AST
   c. Skin biopsy
   d. Schirmer's tear test
   e. Pulmonary function test
   f. Oral exam
   g. Dietician assessment
   h. Gynecological assessment (adult female)

See Section 11 for diagnosis and treatment guidelines for acute and chronic GVHD.

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

Table 7: Post-Transplant Evaluation (See text for pre-transplant evaluations)

This is a recommended evaluation schedule. Note that research draws are for FHCRC patients only. See Table 8 for peripheral blood research studies. See Table 9 for additional lab instructions.

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
CLL	**BM aspirate - unilateral** * After one year from transplant if patient is in CR, bone marrow is recommended only as clinically indicated.									
	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	Clinical	*If abnormal pre-transplant	*see comment	*see comment	*see comment	*see comment	*see comment	*see comment	*see comment
	BM biopsy – unilateral* After one year from transplant if patient is in CR, bone marrow is recommended only as clinically indicated.									
	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 abnormal 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
	B2 microglobulin	Clinical	*If abnormal pre-transplant			*see comment				
	LDH	Clinical				x	x	x	x	x
	Additional research studies	Research – Maloney Lab		See Table 8 for additional peripheral blood research studies						
	Imaging									
	CT of chest, abdomen, pelvis (neck if indicated)	Clinical	* Needed on Day 56 only if abnormal pre-transplant		*see comment	x	x	x	x	x
	GVHD evaluation	Clinical				x				

Table 8: <u>Peripheral blood research studies</u>

Disease	Specimen	Research	Comment	Days												Years	
				10	24	35	38	49	60	63	80	84	110	140	180	1	1.5
CLL	T cell response studies	Research – Maloney Lab	**Only for pts. who have signed Consent D**									x			x	x	
	Polymorphism studies	Research – Maloney Lab	For all Patients									x**					
	Rituxan trough levels*	Research – Maloney Lab	For all Patients	x	x		x										
	Rituxan pharmacokinetics	Research – Maloney Lab	For all Patients						x			x			x	x	

*Rituxan trough level on Day +10, Day +24, and Day +38 should be done immediately prior to rituximab dose

** Polymorphism studies is included with the T-cell response draw, no extra blood will be drawn

Additional information for research studies is as follows:

The blood obtained from the T cell response studies will be used as a source of donor T cells that recognize recipient minor histocompatibility or leukemia associated antigens. Briefly, mononuclear cells (PBMC) will be separated from the blood and aliquots will be cryopreserved. Aliquots of the posttransplant PBMC will be stimulated in vitro with irradiated (3300 rads) recipient PBMC and leukemia cells that were cryopreserved pretransplant (Section 10). The pretransplant recipient PBMC may be activated with CD40L prior to irradiation and use as stimulator cells. Cultures will be restimulated at weekly intervals, supplemented with IL-2 (10-20 U/ml), and assayed for cytolytic and proliferative responses against recipient PHA induced T cell blasts, EBV-LCL, CLL and fibroblasts and against donor target cells generated from the donor PBMC. Independent aliquots of cultures exhibiting responses to recipient CLL but not donor cells will be depleted of $CD4^+$ or $CD8^+$ T cells to determine the subset of cells mediating cytolytic activity. $CD8^+$ and $CD4^+$ T cells from T cell lines that are reactive with the patient CLL will be cloned by limiting dilution and the T cell clones will be further analyzed for specificity and function.
$CD3^+$ $CD8^+$ T cell clones will be tested for cytolytic activity against ^{51}Cr labeled donor and recipient B-LCL, PHA-induced T cell blasts, skin fibroblasts, and recipient CLL. T cell clones that lyse recipient hematopoietic cells but not dermal fibroblasts or donor hematopoietic cells (<5% lysis at E/T of 5:1) will be characterized for the class I MHC restricting allele using a panel of B-LCL from donors that are partially matched at HLA with the recipient. T cell clones specific for minor histocompatibility antigens expressed by CLL and presented by common class I alleles (e.g. HLA A0101, A0201, A0301, A1101, A2402, B0702, B0801, B3501, B4001, and B4402) will be preferentially selected for gene discovery using cDNA expression cloning or genetic linkage analysis.

The blood obtained for the polymorphism studies will be used to determine polymorphisms of FCγRIIIa and CD32 in comparison to pre-transplant polymorphisms.

Table 9: Additional Lab Instructions

Note: All bone marrow tests are done on aspirate unless specifically identified as biopsy.
Off-site providers may use local facilities for the tests. All research studies are for FHCRC patients only.

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
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 Label "protocol 1840)	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
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
ZAP – 70 by Flow cytometry	Clinical	5mL blood in green-top tube	UW Hematopathology Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
T cell response studies	Research	Pretransplant: 10mL blood in red-top tube and if Consent D is signed: 40 mL in green-top tube Label "protocol 1840"	Maloney Lab	FHCRC D1-331 (206) 667-4260
T cell response studies	Research	Posttransplant: 10mL blood in red-top tube and if Consent D is signed: 30 mL in	Maloney Lab	FHCRC D1-331 (206) 667-4260

Specimen / Test	Type	Instructions	Lab Name	Contact Information
		green-top tube Label "protocol 1840"		
Polymorphism studies	Research	10mL blood in green-top tube (only if no T-cell response drawn)	Maloney Lab	FHCRC D1-331 (206) 667-4260
Rituxan trough levels	Research	10mL blood in red-top tube	Maloney Lab	FHCRC D1-331 (206) 667-4260
Rituxan pharmacokinetics	Research	10mL blood in red-top tube	Maloney Lab	FHCRC D1-331 (206) 667-4260

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

C. Donor Evaluation

1. 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.
2. A blood sample (10cc heparinized blood) from related and unrelated donors should be sent to the Maloney lab for determination of patient FCγRIIIa and CD32 polymorphisms prior to HCT.

11. Drugs and toxicities

Detailed treatment-related adverse events will be reported using the adapted NCI Common Toxicity Criteria (Appendix P).

A. TBI

TBI will be given in one 200 cGy fraction from linear accelerator at a rate of 6 - 10 cGy/min. Dosimetry calculations are performed by the radiation therapist. At the dosage of TBI used in this protocol, patients have not experienced the adverse effects associated with myeloablative TBI such as fever, alopecia, parotitis, diarrhea, reversible skin pigmentation, and mucositis. Late effects are unknown but may include cataract formation, growth retardation, pulmonary damage, and carcinogenesis.

B. CSP

See section 9.E.1 for information about administration and dosage adjustments. Side effects are generally reversible and may include renal insufficiency and failure, hypomagnesemia, paresthesias, tremor, seizures, visual disturbances, paresis, disorientation, depression, confusion, somnolence, coma, nausea, hypertension, hemolytic-uremic syndrome, hyperglycemia, gynecomastia, and hypertrichosis.

C. MMF

Mycophenolate mofetil (MMF): is supplied in 250mg hard gelatin capsules. Capsules

may be stored at room temperature.

1. Precautions: MMF has not extensively been studied in patients after HSC transplantation. Previous clinical studies in patients after kidney transplantation suggested that the principal adverse reactions associated with the administration of MMF include leukopenia, sepsis, vomiting and diarrhea. Patients will be monitored for the development of these complications.

2. Adverse Events: Studies in solid organ transplant recipients suggest that MMF may be associated with vomiting, diarrhea, anemia, leukocytopenia and infection. In the setting of marrow transplantation, however, several etiologic factors may contribute to above mentioned symptoms. 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 causative for the abnormality. These adjustments should be discussed with the principal investigator. Dose administration and adjustments are described in Section 9.E.2.

D. 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.

E. Rituximab (rituxan)

Chemistry and Mechanism of Action. The rituximab antibody is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. The antibody is an IgG1 kappa immunoglobulin containing murine light- and heavy-chain variable region sequences and human constant region sequences. Rituximab is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids (based on cDNA analysis) and has an approximate molecular weight of 145 kD. Rituximab has a binding affinity for the CD20 antigen of approximately 8.0 nM.

Stability and Storage: Rituximab solutions for infusion are stable at 2° to 8° C (36° to 46° F) for 24 hours and at room temperature for an additional 12 hours. Rituximab vials should be protected from direct sunlight.

Adverse Events. The predominate toxicity of Rituximab is infusion related fever, chills, rigors, hypotension during the initial infusion. Premedication with acetaminophen and diphenhydramine may decrease the severity of the infusion complex. In the event of an

infusion reaction, the infusion should be slowed or stopped until the symptoms resolve or are successfully treated. The infusion should be restarted at one half the previous rate. Rapid depletion of B-lymphocytes may produce tumor lysis syndrome. Rituximab administration must be followed precisely. Patients on antihypertensive medications, or with prior history of cardiac arrhythmias may require additional clinical monitoring. . Other adverse events of lowering of white blood cell or platelet counts, abnormal heart rhythms and/or congestive heart failure have also occurred. Rituximab treatment may involve risks to the embryo or fetus that are unknown and both women and men should practice contraception. Since Rituximab has only recently been approved by the FDA for use in the treatment of lymphoma, there is no long term information regarding toxicity. Very rarely, in about 1 per 1000 patients treated, severe reactions resulting in death have been associated with Rituximab infusion.

F. GVHD

For related recipients: In our phase I/II trials, grade II-IV acute GVHD associated with primary engraftment occurred in 49% of 192 evaluable patients, and the addition of fludarabine did not influence the incidence of grade II-IV or III-IV acute GVHD. Clinical extensive chronic GVHD occurred in 74 (67%) of 110 evaluable patients. Two of the first 10 evaluable patients receiving an HLA matched sibling donor transplant after conditioning with fludarabine and TBI on Protocol #1533 died of grade IV acute GVHD following discontinuation of CSP at day +56. Fifteen of 21 evaluable patients on Protocol #1533 had to restart immunosuppression after tapering off of CSP for clinically acute GVHD. The median day post-transplant for patients restarting immunosuppression for clinically acute GVHD was day +85 (range 65 to 107). These results prompted revision of the protocol (Protocol #1596) with extension of CSP to day +56 followed by a CSP taper that is adjusted based on the risk of relapse.

For unrelated recipients: After nonmyeloablative HCT from 10/10 HLA-antigen matched unrelated donors, the incidences of grades II, III and IV acute GVHD in patients who received G-PBMC grafts were 42%, 9% and 2% in protocol 1463 (with MMF q12hrs), and 39%, 10%, 2% in protocol 1641 (with MMF q8hrs) respectively. Acute GVHD has been readily controlled in most patients with high dose corticosteroids. Psoralen activated ultraviolet light (PUVA) has been required on occasion. Chronic extensive GVHD has occurred in 40 and 45% of patients in protocols 1463 and 1641, respectively.

1. Diagnosis:

a. On the day of GVHD evaluation, the diagnosis of acute or chronic GVHD will be made based on clinical criteria (Appendix F and G). Skin involvement will be assessed by biopsy and percentage of involved body surface area will be recorded in the medical record and CRF. GI symptoms suspicious for GVHD will be evaluated by biopsy as indicated.

b. If manifestations of GVHD satisfy criteria for acute GVHD (Appendix F) and are not pathognomonic for chronic GVHD (Appendix G), for purposes of documentation and treatment, GVHD will be considered acute. The day post-transplant will not be used to discriminate acute from chronic GVHD. This

recommendation is based on the observation[77][102] that the onset of acute GVHD can be delayed after nonmyeloablative conditioning.

c. If manifestations of GVHD are pathognomonic of chronic GVHD (Appendix G), for purposes of documentation and treatment, GVHD will be considered chronic even if concurrent manifestations satisfy criteria for acute GVHD (Appendix F).

2. Recommended Treatment:

a. Patients developing acute GVHD ≥ grade II while on CSP taper or off immunosuppression:

i. CSP 5.0 mg/kg PO Q12 hrs. If there is concern of GI absorption use IV route (1.5 mg/kg Q12hrs).

ii. Prednisone (2 mg/kg/day) may be added at diagnosis or if the GVHD manifestations are mild when there is no response after 72 hours or if there is progression of GVHD during the 24 hours after the start of CSP. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6 week steroid taper See below section 2.b. for MMF and CSP taper recommendations.

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

b. Patients who develop acute GVHD ≥ grade II prior to day +56 for related and prior to day +100 for unrelated recipients:

Related recipients:

i. Patients who develop acute GVHD ≥ grade II prior to day +28 should receive prednisone (2 mg/kg/day) or intravenous equivalent. MMF should not be discontinued on day +28. If nausea and/or vomiting prevent the oral administration of MMF, MMF should be administered intravenously at 15 mg/kg q8hrs. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6 week steroid taper. MMF may be discontinued without taper, once prednisone has been tapered to a dose of 0.5 mg/kg/day.

ii. Patients who develop acute GVHD ≥ grade II after day +27 should receive prednisone (2 mg/kg/day) or intravenous equivalent. MMF need not be restarted.

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

Unrelated recipients:

i. Patients who develop acute GVHD ≥ grade II prior to day +40 should receive prednisone (2 mg/kg/day) or intravenous equivalent.

Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6 week steroid taper. When steroids are tapered to 0.5 mg/kg p.o. q.d then an MMF taper should be initiated. In the absence of a GVHD flare, the MMF and prednisone tapers should continue until completion. The suggested sequence for tapering MMF and CSP is as follows: taper MMF over 1-2 months, then taper the Cyclosporine 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 MMF, MMF should be administered intravenously at 15 mg/kg TID.

ii. Patients who develop acute GVHD ≥ grade II after day +90 should receive prednisone (2 mg/kg/day) or intravenous equivalent. MMF need not be restarted.

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

c. Patients with clinical extensive chronic GVHD: CSP 5.0 mg/kg PO Q12hrs and prednisone 1 mg/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 ≥ grade II acute GVHD (e.g. erythematous rash, diarrhea, hyperbilirubinemia) **and** are pathognomonic of clinical extensive chronic GVHD (e.g. lichenoid oral changes, ocular sicca, scleroderma, bronchiolitis obliterans, contractures), should receive prolonged immunosuppressive therapy similar to that for chronic extensive GVHD.

G. Myelosuppression

Grade IV myelosuppression will be defined as a decrease in ANC to ≤500/μL and/or platelet count to ≤ 20,000/uL. If myelosuppression occurs, a bone marrow aspirate and biopsy should be performed in the proper clinical circumstances to exclude disease progression or graft rejection. 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). Myelosuppression may occur in this patient population for a number of reasons such as direct toxic effect of drugs, rejection, or relapse.

Patients with myelosuppression may be managed as follows:

1. Suspected MMF toxicity: refer to sections **9.E.2.f** *Guidelines for MMF dose adjustment* for management recommendations.
2. Suspected ganciclovir toxicity: consider changing to foscarnet.

3. Patients who are > 21 days after HCT with a hypoplastic marrow and an ANC of ≤750/uL may receive G-CSF 5μg/kg/day S.C.
4. Thrombocytopenic patients will receive platelet transfusion as per standard care.

12. Toxicity Reporting Guidelines

Detailed treatment-related adverse events will be reported using the adapted NCI Common Toxicity Criteria (Appendix P).

13. 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.

Collection of Survival and Disease Response Data

Centers enrolling patients will complete case report forms within 120 days of HCT that detail events occurring within the 100 days after HCT (Appendix M). The case report form provides detailed descriptions of the transplantation procedure, disease response, and complications. Detailed treatment-related adverse events will be reported using the NCI Common Toxicity Criteria (Appendix P). The coordinating center will contact subjects when they return to the HCT center for routine HCT follow-up at 3, 6, 12 and 24 months to ascertain the patient's disease status and survival. Referring physicians are required to report the date and cause of death within 48 hours of occurrence. Supporting medical records must be available upon request to substantiate data on the case report forms. Death information including date and cause will be reviewed from these medical records, and will be confirmed by death certificate.

14. Statistical Consideration and Criteria of Termination of Study

The primary objective of this protocol is to estimate survival using allogeneic HCT from related or unrelated donors after nonmyeloablative conditioning and peri-transplant Rituximab in patients with advanced CLL, and to gain a preliminary indication as to whether survival is improved compared to treatment with conventional salvage therapies. The primary endpoint will be survival at 18 months. The 18 months time point is chosen because prior experience with HCT indicates that survival

reaches a relative plateau by that time, and thus offers an endpoint that can be evaluated somewhat more rapidly than 2 year survival. The estimated 18 months survival with standard treatment (Campath-1H) from historical patients is approximately 0.45. This treatment approach will be deemed promising for further study if we can achieve reasonable confidence that survival exceeds 0.45. Reasonable confidence will be taken to mean that the lower limit of a one-sided 90% confidence interval for the true survival fraction at 18 months is greater than 0.45.

Protocol 1840 has enrolled 14 patients so far before the current changes. We anticipate enrolling eighty new patients in eight years from the data when peri-transplant Rituximab was added to the inclusion criteria. The total targeted accrual for the protocol will be 94 but only the last 80 patients will be evaluated for the study objectives.

Eighty new patients will be evaluated; thus the above criterion will be met if 43 or more of 80 patients are alive at 18 months, then we will be at least 90% confident that the true survival rate exceeds 0.45. We assume based on our prior studies that we will achieve 100% ascertainment of this endpoint in these closely followed patients. The probability of such a favorable outcome is 89%, if the true survival fraction at 18 months is 60% and 63% if the true fraction is 55%. Our current experience with non-myeloablative HCT from unrelated donor in CLL indicates that this is a reasonable expectation.

A secondary objective of this protocol is to test the hypothesis that pre- and post-transplant rituximab will reduce the incidence of chronic GVHD. Therefore, the incidence of chronic GVHD in the study population will be compared to that seen in our published historical controls receiving non-myeloablative allogeneic HCT without rituximab. Enrollment of a total of 80 new patients will yield 90% power to detect an absolute decrease of 15% in the risk of chronic GVHD (45%→30%), and 64% power to detect a 10% absolute risk reduction for chronic GVHD (45%→35%), at the 1-sided 0.1 level of significance.

Criteria for Termination of Study

This will be a single stage trial of a single cohort of 80 patients. However, safety stopping rules will be imposed for acute grade IV GHVD at anytime that precludes the diagnosis of chronic GVHD, and transplant-related mortality at 200 days. If sufficient evidence exists to suggest that the true rate of transplant related mortality at day 200 exceeds 0.30, or the true rate of grade IV acute GVHD exceeds 0.10, then the protocol will be terminated. Sufficient evidence will be taken to be any observed rate for which the lower limit of a one-sided 80% confidence interval exceeds the target rate.

Therefore, **stopping rules** will be imposed for:

- Transplant-related mortality at day 200 > 30%
- Grade IV acute GVHD >10% at anytime that precludes the diagnosis of chronic GVHD

These rules will be evaluated after every tenth patient is evaluable for the endpoint in question. Operationally, this will occur:

- Transplant-related mortality (by day 200. 30% threshold): 3/5, 5/10, 7/15, 9/20, 10/25, 12/30, 14/35, 15/40, 19/50, 22/60, 25/70, 28/80
- Grade IV acute GVHD (10% threshold) at anytime that precludes the diagnosis of chronic GVHD: 2/5, 3/10, 3/15, 4/20, 5/25, 5/30, 6/35, 7/40, 8/50, 9/60, 10/70, 11/80.

These stopping criteria are listed in Table 10. Patients may continue to be enrolled pending the evaluation of these stopping rules after every tenth patient, but the outcome in subsequent patients cannot be used to override a stopping rule triggered in an earlier number. The operating characteristics of these stopping rules are given in Table 11.

Table 10. Criteria for stopping trial based on day 200 evaluation

Number of patients enrolled	Number of Patients with Grade IV GVHD	Number of Patients Experiencing day-200 TRM
10	3	5
20	4	9
30	5	12
40	7	15
50	8	19
60	9	22
70	10	25
80	11	28

Table 11. Operating characteristics of stopping rules

Day 200 TRM			Grade IV acute GVHD at anytime that precludes the diagnosis of chronic GVHD		
True rate of event	Probability of stopping*	Average N at stopping*	True rate of event	Probability of stopping*	Average N at stopping*
0.35	70%	46	0.15	77%	44
0.40	91%	32	0.20	96%	28
0.45	98%	23	0.25	>99%	19

15. Targeted Planned Enrollment Table

Table 12 shows the anticipated distribution of subjects based on data from the US Census Bureau and the age-adjusted incidence of CLL in populations in the United States from SEER.

Table 12. Targeted Planned Enrollment Table

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex / Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	1	2	3
Not Hispanic or Latino	32	45	77
Ethnic Category Total of All Subjects*	33	47	80
Racial Categories			
American Indian / Alaska Native		1	1
Asian	1	1	2
Native Hawaiian or Other Pacific Islander			
Black or African American	1	1	2
White	31	44	75
Racial Categories: Total of All Subjects*	33	47	80

*The "Ethnic Category Total of All Subjects" must be equal to the "Racial Categories Total of All Subjects". This is the targeted planned enrollment after adding peri-transplant rituximab and allowing grafts from related or unrelated HLA-matched donors).

16. Data Safety Monitoring Board and Adverse Events Reporting

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

This protocol is a multi-institutional clinical trial that is monitored by the principal investigators (PI), Drs. Sorror and Dr. Maloney, with oversight by Dr. Sandmaier, and Dr. Storb, a Data Safety and Monitoring Board (DSMB), the Protocol and Data Monitoring Committee (PDMC) and the Institutional Review Board (IRB). The PI reviews outcome data with the protocol mentors for each local individual patient on a weekly basis at a minimum of 3 months after HCT and the updated data are presented at Mixed Chimerism Meetings that includes peer review by co-investigators.

Adverse events are reported to the trial coordinator, one of the study nurses, or directly to the PI. The trial coordinators at collaborating centers or the local PIs will fax an official report of a serious adverse event to the coordinating center (FHCRC) within ten days. The serious adverse event report is reviewed by Dr. Sorror and Dr. Maloney. If the serious adverse event meets the FHCRC and/or NCI criteria for 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. This protocol has a dedicated independent DSMB responsible for monitoring patient safety on this clinical trial. The DSMB meets twice a year and all outcome data is reviewed including all adverse events reported to the coordinating center (FHCRC) along with those officially reported to the FHCRC IRO. The DSMB confirms whether or not 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. Furthermore, the FHCRC also has a PDMC that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. An initial IRB review and approval will occur before any patients are enrolled in the trial and an annual IRB review and approval also is required. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual.

With respect to safety, all patients are monitored for the development of GVHD, myelosuppression, infections, and organ-specific toxicities. All patients, regardless of diagnosis, will be considered in the safety analysis. Because of the older age profile of the patients, complications of HCT, GVHD and infections, may be less well tolerated than patients on other protocols. These events will be closely monitored and severity of GVHD graded. Formal stopping rules for TRM, defined as death before day 100 not related to progression of disease, will be closely monitored and stopping rules are provided above in **section 14** Statistical Consideration and Criteria Termination of Study. This endpoint encompasses serious problems associated with the potential complications of severe GVHD, infections, and rejection.

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). Drs. Sorror and Maloney will review the official CRF and primary source documents. When the CRFs are verified, they are signed by PI. Thus, multiple health care providers provide independent

observations and participate in monitoring this trial. Drs. Sorror or Maloney 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. In addition, direct oversight from other PIs including Dr. Sandmaier and Dr Storb, will further minimize potential conflict of interest inherent in the PI serving as primary study monitor. 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.

This protocol is a multi-institutional protocol and all collaborating centers sign an agreement with the FHCRC stating that data generated from patients from the protocol will be reported accurately in a timely manner to the FHCRC. All centers have an IRB that review the protocol and that the local PIs contact when an adverse event on the protocol occurs. Most of the centers have internal auditing mechanisms that assure accurate assessment of clinical outcomes. Clinical outcome data are summarized and transmitted from collaborating centers as CRFs. When possible, primary source documents regarding patient outcomes are collected with patients' names removed and replaced by Unique Patient Numbers (UPNs). The CRFs are generated from the collaborating centers at defined time points (100 days, 6 months, and yearly). The local PI will review the official CRF and primary source documents. When the CRFs are verified, they are signed by the local PI and the data are entered into a central database managed by the Trial Coordinator.

B. Plans for assuring compliance with requirements for reporting adverse events

The adverse event reporting in this multi-institution clinical trial will follow the FHCRC Guidelines for serious adverse event (SAE) reporting. The FHCRC guidelines have been expanded to give further details on what events meet expedited reporting requirements for consistency in SAE reporting across the centers. Definitions of particular events that require reporting are in Appendix P. These guidelines (attached in Appendix I.) detail the expedited reporting requirements, definitions of particular events. 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. Maloney and Dr. Sorror. If the SAE meets the FHCRC criteria for expedited reporting then an official signed report is submitted to the FHCRC Institutional Review Office (IRO) within 10 days. All deaths, regardless of the cause, are reported to the IRB. 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. Furthermore, an additional safeguard for adverse event analysis and reporting in this protocol is provided by stopping rules. All collaborating PIs have fulfilled all NIH requirements for training in human subjects' protection.

C. 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 PDMC, of this clinical research trial will be reported to the NCI grant program director by the PI.

D. 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.

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APPENDICES – TABLE OF CONTENTS

APPENDIX A

ELIGIBILITY GUIDELINES FOR DONOR PBSC APHERESIS FOR TRANSFUSION

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

APPENDIX B

THE KARNOFSKY PERFORMANCE STATUS SCALE

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

APPENDIX C
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.

Appendix C (continued)

MAJOR ABO INCOMPATIBLE				
Recipient anti-Donor titer	≥ 1:32	<20ml RBC total	⟹	Infuse without modification
		>20ml RBC total	⟹	RBC depletion of component
	≤ 1:16		⟹	Infuse without modification
MINOR ABO INCOMPATIBLE				
Donor anti-Recipient titer	≥ 1:256	Plasma depletion of component		
	≤ 1:128	Infuse without modification		

APPENDIX D
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

CMV Prevention: Surveillance and Preemptive Therapy

CMV Prevention

CMV Disease: Diagnosis and Treatment

CMV Disease Treatment

Antifungal Therapy Guidelines

Antifungal Therapy

Pneumonia / Pneumocystis Jiroveci Prophylaxis

pneumocystisjiroveci

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

AntibioticProphylaxis -Encapsulated

Vaccinations

Vaccines

Foscarnet

Foscarnet

APPENDIX E

GRAFT-VERSUS-HOST DISEASE DATA COLLECTION FORM

GVHD TREATMENT:

Check each agent used to treat GVHD: Date Treatment Started ☐ None

- ☐ Cyclosporine __ __/__ __/__ __ __ __
- ☐ Systemic Corticosteroids __ __/__ __/__ __ __ __
- ☐ Topical Corticosteroids __ __/__ __/__ __ __ __
- ☐ Beclomethasone __ __/__ __/__ __ __ __
- ☐ Rapamycin __ __/__ __/__ __ __ __
- ☐ MMF __ __/__ __/__ __ __ __
- ☐ ATG __ __/__ __/__ __ __ __
- ☐ In Vivo anti-T lymphocyte McAb __ __/__ __/__ __ __ __
- ☐ Blinded Randomized Trial __ __/__ __/__ __ __ __
  Specify_____
- ☐ Other, specify_____ __ __/__ __/__ __ __ __
  _____

Date of Death: __ __/__ __/__ __ __ __

Was an autopsy done? ☐ Yes ☐ No

Was GVHD found at autopsy? ☐ Yes ☐ No ☐ N/A

If yes:
- ☐ Skin
- ☐ Liver
- ☐ Gut

GVHD SUMMARY

Date of onset: __ __/__ __/__ __ __ __ Day_____

Overall Grade_____

Skin_____ Liver_____ Gut_____

Comments_____
_____
_____

BIOPSIES

Was skin biopsy done? ☐ Yes ☐ No
Date of skin biopsy: __ __/__ __/__ __ __ __
Results:
- ☐ Negative
- ☐ Positive GVHD
- ☐ Positive Other

Was liver biopsy done? ☐ Yes ☐ No
Date of liver biopsy: __ __/__ __/__ __ __ __
Results:
- ☐ Negative
- ☐ Positive GVHD
- ☐ Positive Other

Did patient have **any** GI Biopsy? ☐ Yes ☐ No
Date of biopsy: __ __/__ __/__ __ __ __
Results:
- ☐ Negative
- ☐ Positive GVHD
- ☐ Positive Other

CLINICAL SKIN

Did patient ever have rash after transplant? ☐ Yes ☐ No

Was rash thought to be due to GVHD? ☐ Yes ☐ No

Date of onset of rash: __ __/__ __/__ __ __ __

Extent of rash on date of first treatment for GVHD: _____
Maximum severity of rash:
- ☐ 0 – No rash
- ☐ 1 – Maculopapular rash <25% BSA
- ☐ 2 – Maculopapular rash 25-50% BSA
- ☐ 3 – Generalized erythroderma
- ☐ 4 – Generalized erythroderma with bullous formation and desquamation

Date of maximum rash severity: __ __/__ __/__ __ __ __

CLINICAL LIVER

Bilirubin level on date of first treatment for GVHD: _____

Maximum Bilirubin Date __ __/__ __/__ __ __ __

- ☐ 0 – Bilirubin < 2.0 mg/dl
- ☐ 1 – Bilirubin 2.0 – 3.0 mg/dl
- ☐ 2 – Bilirubin 3.1 – 6.0 mg/dl

☐ 3 – Bilirubin 6.1 – 15.0 mg/dl
☐ 4 – Bilirubin >15.0 mg/dl

Did patient have other liver process? ☐ Yes ☐ No
☐ VOD
☐ Cyclosporine Toxicity
☐ Sepsis
☐ Other liver complication

CLINICAL GUT

GI consult? ☐ Yes ☐ No Date of Consult:__ __/__ __/__ __ __ __

Stool volume on date of first treatment for GVHD: _____

Maximum Daily Stool Output after day 21

☐ 0 - 0 - ≤200 ml
☐ 1 - >200 but ≤1000 ml
☐ 2 - >1000 but ≤1500 ml
☐ 3 - >1500 ml
☐ 4 - Severe abdominal pain with or without ileus

Date of maximum stool output: __ __/__ __/__ __ __ __

Was stool mixed with urine on day of Maximum Stool Output? ☐ Yes ☐ No
If stool volume not measurable, what was maximum number of stools/day? _____
Date of maximum number of stools/day: __ __/__ __/__ __ __ __
Did patient have the following process?
☐ Frank blood in stool attributed to GVHD
☐ CMV infection of GI tract at time of maximum stool volume
☐ Narcotic withdrawal at time of maximum stool volume
☐ C. difficile infection at time of maximum stool volume
☐ Other GI process Specify_____

Date Completed __ __/__ __/__ __ __ __ Completed by: _____

APPENDIX F
ACUTE GRAFT-VERSUS-HOST DISEASE GRADING[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 GRADING [a]

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. 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 systems as listed below.

Skin	Erythema, dryness, pruritus, pigmentary changes (i.e. hyperpigmentation, vitiligo), mottling, papulosquamous plaques, nodules, exfoliation, macular-papular or urticarial rash, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)
Nails	Ridging, onychodystrophy, onycholysis
Hair	Premature graying, (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair
Mouth	Dryness, burning, gingivitis, mucositis, striae, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tooth decay, tightness around the mouth
Eyes	Dryness, burning, blurring, gritty eyes, photophobia, pain
Vagina/vulva	Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not included
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase ≥ 3x upper limit of normal, AST or ALT ≥4x upper limit of normal or total serum bilirubin ≥ 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures

Serositis	Chest pain or cardiopulmonary comprise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

Laboratory testing sand diagnostic indicators of chronic GVHD[a]

Eye	Schirmer's test with a mean value ≤ 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as 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. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans o the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/µl), eosinophilia, hypogammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

[a] From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

GRADING OF CHRONIC GRAFT-VERSUS-HOST DISEASE

A Clinical limited chronic GVHD

1. Oral abnormalities consistent with chronic GVHD, a positive skin or lip biopsy, and no other manifestations of chronic GVHD
2. Mild liver test abnormalities (alkaline phosphatase ≤ 2x upper limit of normal, AST or ALT ≤ 3x upper limit of normal and total bilirubin ≤ 1.6) with positive skin or lip biopsy, and no other manifestation of chronic GVHD
3. Less than six papulosquamous plaques or limited skin rash or dyspigmentation (< 20% of the body surface), positive skin biopsy, and no other manifestations of chronic GVHD
4. Ocular sicca (Schirmer's test ≤ 5mm), positive skin or lip biopsy, and no other manifestations of chronic GVHD
5. Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of chronic GVHD

B. Clinical extensive chronic GVHD

1. Involvement of two or more organs with symptoms or signs of chronic GVHD, with biopsy documentation of chronic GVHD in any organ
2. > 15% base line body weight loss not due to other causes, with biopsy documentation of chronic GVHD in any organ
3. Skin involvement more extensive than defined for limited chronic GVHD, confirmed by biopsy
4. Scleroderma or morphea
5. Onycholysis or onychodystrophy thought to represent chronic GVHD, with documentation of chronic GVHD in any organ
6. Decreased range of motion in wrist of ankle extension due to fasciitis caused by chronic GVHD
7. Contractures thought to represent chronic GVHD
8. Bronchiolitis obliterans
9. Positive liver biopsy; abnormal liver function tests not due to other causes with alkaline phosphatase > 2x upper limit of normal, AST or ALT > 3x upper limit of normal, or total bilirubin > 1.6, and documentation of chronic GVHD in any organ
10. Pericarditis or pleuritis not due to other causes
11. Positive upper or lower GI biopsy

[a] From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

APPENDIX H

EVALUATION OF DISEASE RESPONSE FOR CLL

CLL: Modified response criteria based on the NCI-Working Group and the International Workshop Group Joint Formal Criteria for evaluating disease response for CLL. [1-3]

Complete Remission (CR)	
Imaging studies (Xray, CT, MRI) (nodes, liver, and spleen)	Normal
Peripheral blood by flow cytometry	No clonal lymphocytes
Bone marrow by morphology	No nodules; or if present, nodules are free from CLL cells by immunohistochemistry
Duration	≥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 (PR):	
Both criteria:	
Absolute lymphocyte count in peripheral blood	≥50% decrease[3]
Physical exam/Imaging studies (nodes, liver, and/or spleen)	≥50% decrease[3,4]
Duration	≥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
Lymph node Biopsy	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.	

[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
INCLUDING PROCEDURE FOR REPORTING ADVERSE EVENTS

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 and a copy of the signed informed consent will be faxed (206-667-5378) prior to treatment initiation. Patients must be registered prior to treatment initiation for valid registration

Appendix I (continued)

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 unexpected and serious adverse events which may be due to study treatment or intervention must be reported to the FHCRC Institutional Review Office as soon as possible but within at least 10 calendar days of the investigator learning of the event.

Definitions

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

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction. Study toxicities are graded using the NCI Common Toxicity Criteria (where appropriate use the criteria for transplant patients.) All Grade 4 (life-threatening) toxicities occurring between start of conditioning to day 200 that meet expedited reporting requirements must be reported as soon as possible but within at least 10 calendar days of the investigator learning of the event.

Unexpected Adverse Event – An adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product). If applicable product information is not available, such as for studies that do not involve pharmaceutical products or devices, an unexpected adverse event is an adverse event that was not described in the study protocol or informed consent.

Serious Adverse Event (SAE) – Any adverse event occurring that results in any of the following outcomes:

- Death – start of conditioning to day 200, regardless of cause,
- A life-threatening adverse event (see above)
- A persistent or significant disability/incapacity,
- A congenital anomaly
- Requires intervention to prevent permanent impairment or damage.

Appendix I (continued)

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 nonmyeloablative 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.

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.

Attribution - The FHCRC designation for the determination of whether an adverse event is related to a medical product, treatment or procedure will be as follows:

- Related – includes adverse events that are definitely, probably, or possibly related to the medical treatment or procedure.
- Not Related – includes adverse events are doubtfully related or clearly not related to the medical treatment or procedure.

The FHCRC Serious Adverse Event (SAE) Report Form should be completed for all adverse events that meet the expedited reporting requirements. All available information should be submitted but it is acceptable to fax an incomplete report form at the initial report. A completed report should be faxed as soon as possible but must be received within 10 calendar days.

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.

Serious adverse events that do not meet the requirement for expedited reporting (not related to study treatment or expected) will be reported to the IRB as part of the annual renewal of the protocol.

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.

Appendix I (continued)

Procedure for Reporting Serious and Unexpected Adverse Events from Participating Sites
Regulations defining the responsibilities for reporting serious and unexpected adverse reactions are defined above. Serious and unexpected adverse events must be reported to the FHCRC Investigator within 10 days of learning of the event. This includes patient deaths (serious, unexpected, and related/possibly related), regardless of cause, occurring start of condition to day 200 post-transplant procedure. The immediate telephone report must be followed by faxed comments to the 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.

Obligation of Investigators
All grade 3 or 4 adverse events (or highly unusual grade 2 adverse events), which occur between start of conditioning to day 100 during the study will be recorded on the Case Report Form (**Appendix M)**. These adverse events which are observed by the Investigator or reported by the patient, whether or not attributed to the study, will be reported on the Case Report Form using the modified (for HSCT) NCI Common Toxicity Criteria (**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).

Adverse events will be graded accordingly: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life threatening or debilitating, and 5 = fatal. All Grade 4 (life-threatening) or Grade 5 (fatal) events on the NCI scale meet expedited reporting requirements.

Association or relatedness to the study agent will be graded as follows: 1 = unrelated, 2 = unlikely, 3 = possibly, 4 = probably, and 5 = definitely related.

V. Case Report Forms

Case report forms must be completed for all patients registered onto the protocol and submitted to the FHCRC data coordinating center. The first case report form (day 28) is due on day 50. For outside centers a Staging Form must accompany the form with the patient staging at registration, day 28, day 56, day 84 and day 100. Staging forms should also be completed with each Follow Up Form completed on day 180, 1 year, 1.5 years, 2 years, 3 years, and yearly thereafter. For Outside Centers, case report forms are expected to be submitted no later than 30 days following the scheduled follow up date.

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.

Appendix I (continued)

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 study staff became aware of event: _____

Date of Report:
☐ Initial Report _____ ☐ ~~Follow-Up~~ Report # _____ ☐ Other

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

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

#1 _____ #2 _____

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

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

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

Report Completed by: _____ Date: _____

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

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

_____ _____
Signature of Principal Investigator Date:

Fred Hutchinson Cancer Research Center

SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ Date of Report: _____

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

APPENDIX K
NOTICE OF DEATH

Patient ID: _____ Date of Death: _____

Place of Event: _____

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

Form completed by: _____Date: _____

APPENDIX L

Patient Demographics and Eligibility Form

1840

Please Fax this completed form to (206)-667-5378 for patient registration.
Questions regarding eligibility should go to Mohamed Sorror, M.D., 206-667-2765.

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 research subject 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 research subject 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).*
- ☐ **Native Hawaiian/Pacific Islander** *(A person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands).*
- ☐ **Black/African American** *(A person having origins in any of the black racial groups of Africa).*
- ☐ **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

Protocol 1840 Eligibility

Inclusion Criteria:
All of the following questions must be marked "Yes" for the patient to enter the study.

1. ☐ Yes ☐ No Patient signed IRB approved consent form. Date: _____
IRB File Number: _____Date of IRB Approval: _____

2. ☐ Yes ☐ No Patient has a diagnosis of CLL (or small lymphocytic lymphoma) or Diagnosis of CLL that progresses to prolymphocytic leukemia (PLL).

3. ☐ Yes ☐ No Patient has at least one of the following:

   a. ☐ Yes ☐ No Has B-Cell CLL or PLL and failed to meet NCI Working Group criteria (Appendix H) for complete or partial response after 2 cycles of therapy with a regimen containing fludarabine (or another nucleoside analog, e.g. 2-CDA, pentostatin) or disease relapse within 12 months after completing therapy with a fludarabine (or another nucleoside analog) containing regimen.

   b. ☐ Yes ☐ No Has B-Cell CLL or PLL and failed FCR or PCR combination chemotherapy at any time point.

   c. ☐ Yes ☐ No Has B-Cell CLL or PLL and had de novo or acquired "17p deletion" cytogenetic abnormality. Patients should have received induction treatment but could be transplanted in 1st CR.

4. ☐ Yes ☐ No Related donor who is genetically phenotypically HLA-identical

   OR

   Unrelated donors who are prospectively:
   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 ☐N/A Have a negative anti-donor cytotoxic crossmatch. Cytotoxic crossmatch **not done as patient and donor are phenotypically identical by molecular methods.**

iv) ☐ Yes ☐ No Patient and donor pairs must not be homozygous at a mismatched allele.

5. ☐ Yes ☐ No Patient is 18 years of age or older.

Exclusion criteria:

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

6. ☐ Yes ☐ No Has active CNS involvement with CLL. For LP requirement, see Appendix N.

7. ☐ Yes ☐ No Patients with active non-hematologic malignancies (except non-melanoma skin cancers).
This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy

8. ☐ Yes ☐ No Patients with a history of non-hematologic malignancies (except non-melanoma skin cancers) currently in a complete remission, who are less than 5 years from the time of complete remission, and have a >20% risk of disease recurrence.
This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy

9. ☐ Yes ☐ No ☐ NA Fertile man or woman unwilling to use contraceptive techniques during and for 12 months following treatment.

10. ☐ Yes ☐ No ☐ NA Female who is pregnant or breastfeeding.

11. ☐ Yes ☐ No The addition of cytotoxic agents for "cytoreduction" with the exception of tyrosine kinase inhibitors (such as imatinib mesylate), cytokine therapy, hydroxyurea, low dose cytarabine, chlorambucil, or rituxan within three weeks of the initiation of conditioning.

12. Patient has organ dysfunction as described below. Please check ***YES*** if patient has any of the following.

a. **Cardiovascular:**

☐ Yes ☐ No Cardiac Ejection Fraction <40%. Ejection fraction is required if age >50 years or there is a history of prior transplant, anthracycline exposure or history of cardiac disease.

☐ Yes ☐ No Poorly controlled hypertension despite multiple antihypertensives.

b. **Pulmonary:**

☐ Yes ☐ No DLCO < 40%, TLC <40%, FEV1 <40% and/or is requiring supplementary continuous oxygen, or severe deficits in pulmonary function testing as defined by pulmonary consultant service. The FHCRC PI of the study must approve of enrollment of all patients with pulmonary nodules.

PI Signature: _____ Date: _____

c. **Hepatic**

☐ Yes ☐ No 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, hepatic damage with bridging fibrosis, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3 mg/dl, or symptomatic biliary disease.

13. ☐ Yes ☐ No Performance status:
    a. Karnofsky score < 60 (see **Appendix B**) for adult patients

14. ☐ Yes ☐ No Infection with HIV

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

Note: The HCT-CI score is:_____
(fax HCT-CI worksheet with registration—see Appendix Q)

Signature of person completing form: _____ Date: _____

Signature of Principal Investigator: _____ Date: _____

APPENDIX M
CORE CASE REPORT FORMS

CRFs

APPENDIX N

INTRATHECAL THERAPY ADMINISTRATION


IntrathecalTherapy.
pdf

Appendix O

HLA Matching Requirements For Unrelated Donors At The SCCA/Fred Hutchinson Allied System

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

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

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

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

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

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

HLA Matching Requirements For Unrelated Donors At The SCCA/Fred Hutchinson Allied System

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

Allele-match for HLA-A, B, C, DRB1 and DQB1.
Single allele disparity for HLA-A, B, C, or DRB1 or DQB1
Two allele disparities for HLA-A, B, or C.
Single allele disparity for HLA-DRB1 and/or a single DQB1 antigen or allele disparity.
Single antigen plus single allele disparity for HLA-A, B, or C.

The following levels of patient-donor mismatch should be restricted to research protocols:

Two antigen disparity, either HLA-A plus C or HLA-B plus C.
Single antigen disparity for HLA-DRB1 with or without DQB1 allele or antigen disparity
Combined disparity of class I and class II loci, i.e. disparity for HLA-A, or B, or C, and any additional disparity for DRB1 or DQB1

Donor Exclusion Criteria to be considered for protocols or standard treatment plans include:

Double locus disparity. Two disparities are not allowed when they both involve the same locus, i.e., the patient is A*0101, A*0201 and the donor is A*0102 and A*0205.
Recipient and donor homozygous at mismatched locus. Patient and donor pairs homozygous at the mismatched locus are considered a two-locus mismatch, i.e., the patient is A*0101 and the donor is A*0201, and this type of mismatch is not allowed.
Recipient homozygous at mismatched locus. If the recipient is homozygous at HLA-A, B, or C and the donor is mismatched at that locus, i.e., patient is A*0101 and donor is A*0101 and A*0201, the risk of rejection is increased. Such a donor should be avoided if there is already an appreciable risk of rejection, i.e., in patients with CML/MDS/Severe Aplastic Anemia (SAA) or those receiving reduced conditioning.

Relevance of HLA matching for transplantation of unrelated hematopoietic cells:
Human Leukocyte Antigen (HLA) typing of patients and prospective hematopoietic stem cell (HSC) donors is carried out to identify and match for HLA determinants associated with successful HSC transplant outcome. While several preliminary studies (1, 2, 3) suggested the importance of allele level matching in hematopoietic cell transplantation (HCT), recent comprehensive studies confirmed that allele-level typing and matching is necessary to optimize clinical outcome in hematopoietic cell transplantation (4, 5, 6, 7).

The pervasiveness of occult HLA mismatch was shown by Petersdorf, et al in an analysis of 300 CML/CP unrelated donor-recipient pairs matched for HLA-A and B by serologic typing, and matched for the DRB1 alleles.(4) The percent of patient-donor pairs found to be matched at the allele level for all 5 loci (HLA-A, B, C, DRB1, DQB1) was only 47% (n=142). High resolution typing demonstrated previously undetected mismatches in 53% (158), indeed 26% (79) pairs were mismatched for multiple alleles. Mismatch of class I HLA was found at one locus in 55 pairs (18%) and at two or more loci in 35 pairs (12%). A single mismatch of class II HLA was

detected in 24 pairs (8%), whereas 7 pairs (2%) had multiple class II mismatches, and 37 pairs (12%) had multiple mismatches involving both class I and class II. These data show the

HLA Matching Requirements For Unrelated Donors At The SCCA/Fred Hutchinson Allied System

importance of high resolution typing for defining the degree of mismatching between potential unrelated patient-donor pairs.

The degree of HLA mismatch, as well as the locus of mismatch, influence the development of alloimmune reactions and have significant implications for the outcome of HSC transplants. Studies of patient-donor pairs have shown an increased risk for graft failure with multiple mismatches that involve at least one class I allele. The incidence of graft failure was 29% in pairs where the mismatch involved more than one class I allele mismatch and 12% for mismatches involving both class I and class II alleles, compared with 2% or less for pairs with either no mismatch or mismatch confined to a single HLA-A, B, C, DRB1 and DQB1 allele. The risk of developing grades III-IV acute GVHD also has been shown to be influenced by the number and class of mismatched alleles. In studies involving primarily Caucasian patient-donor pairs, the highest risk for severe acute GVHD was observed for multiple mismatches involving both class I and class II alleles (2.0 hazard ratio and p=0.02). Pairs with a single class I mismatch did not have a significant increase in acute GVHD compared with matched recipients, but a single class II mismatch or multiple class I mismatches both appeared to confer a higher (though not significant) hazard of severe GVHD. As results of future studies further define risks of mismatches, particularly in nonCaucasian populations, we may be able to delineate more precisely “low risk” from “high risk” mismatches. Until then, the donor selection process should endeavor to identify the best matched donor within the time allowed by the clinical situation.

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HLA Matching Requirements For Unrelated Donors At The SCCA/Fred Hutchinson Allied System

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Approved by the Standard Practice Committee January 16, 2002 (Donor Selection Matching Requirements.doc)
HLA_matching_for_URD_selection.doc
Approved by the Medical Advisory Committee (Medical Exec. Comm.) April 9, 2002
Approved by the Clinical Sectional Meeting April 16, 2002
Revised by the Standard Practice Committee June 15, 2005
Minor wording change approved by SPC chairperson March 13, 2006

APPENDIX P
Adapted from
COMMON TOXICITY CRITERIA (CTC)

Grade		
Adverse Event	**3**	**4**
ALLERGY/IMMUNOLOGY		
Allergic reaction/hypersensitivity (including drug fever)	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis
Vasculitis	Requiring steroids	Ischemic changes or requiring amputation
Allergy/Immunology – Other (Specify,_____)	Severe	Life-threatening or disabling
BLOOD/BONE MARROW		
Bone marrow cellularity		
Hemolysis (e.g., immune hemolytic anemia, drug-related hemolysis, other)	Requiring transfusion and/or medical intervention (e.g., steroids)	Catastrophic consequences of hemolysis (e.g., renal failure, hypotension, bronchospasm, emergency splenectomy)
For BMT studies, if specified in the protocol. For pediatric BMT studies, if specified in the protocol.	>4 u pRBC in 24 hours >30mL/kg in 24 hours	Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin
Grade		
Adverse Event	**3**	**4**
CARDIOVASCULAR (ARRHYTHMIA)		
Cardiovascular/Arrhythmia -Other (Specify, _____)	Symptomatic, and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
CARDIOVASCULAR (GENERAL)		
Acute vascular leak syndrome	Respiratory compromise or requiring fluids	Life-threatening; requiring pressor support and/or ventilatory/support
Cardiac-ischemia/infarction	Angina without evidence of infarction	Acute myocardial infarction

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
CARDIOVASCULAR (GENERAL) continued.		
Cardiac left ventricular function	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Cardiac troponin I (cTnI)	Levels consistent with unstable angina as defined by the manufacturer	Levels consistent with myocardial infarction as defined by the manufacturer
Cardiac troponin T (cTnT)	≥ 0.1 - <0.2 ng/mL	≥ 0.2 ng/mL
Hypotension	Requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	Shock (associated with acidemia and impairing vital organ function due to tissue hypoperfusion)
Myocarditis	CHF responsive to treatment	Severe or refractory CHF
Pericardial effusion/ pericarditis	With physiologic consequences	Tamponade (drainage or pericardial window required)
Syncope (fainting) is graded in the NEUROLOGY category.	-	-
Thrombosis/embolism	Deep vein thrombosis, requiring anticoagulant therapy	Embolic event including pulmonary embolism
Vein/artery operative injury is graded as Operative injury of vein/artery in the CARDIOVASCULAR (GENEARL) category.		
Cardiovascular/General – Other (Specify, _____)	Severe	Life-threatening or disabling

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
COAGULATION		
DIC (disseminated intravascular coagulation) Also consider Platelets. Note: Must have increased fibrin split products or D-dimer in order to grade as DIC.	Laboratory findings present with no bleeding	Laboratory findings and bleeding
Coagulation - Other (Specify, _____)	Severe	Life-threatening or disabling
CONSTITUTIONAL SYMPTOMS		
Weight gain associated with Veno-Occlusive Disease (VOD) for BMT studies, if specified in the protocol. Also consider Ascites Edema, Pleural effusion (non-malignant).	>10% or as ascites	>10% or fluid retention resulting in pulmonary failure
DERMATOLOGY/SKIN		
Erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis)	Severe or requiring IV fluids (e.g., generalized rash or painful stomatitis)	Life-threatening (e.g., exfoliative or ulcerating dermatitis or requiring enteral or parenteral nutritional support)

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
DERMATOLOGY/SKIN continued		
Rash/desquamation associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	Symptomatic generalized erythroderma or symptomatic macular, papular or vesicular eruption, with bullous formation, or desquamation covering ≥50% of body surface area	Generalized exfoliative dermatitis or ulcerative dermatitis or bullous formation
GASTROINTESTINAL		
Ascites(none-malignant)	Symptomatic, requiring therapeutic paracentesis	Life-threatening physiologic consequences
Colitis Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Melena/GI bleeding, Rectal bleeding/hematochezia, Hypotension.	Abdominal pain, fever, change in bowel habits with ileus or peritoneal signs, and radiographic or biopsy documentation	Perforation or requiring surgery or toxic megacolon
Diarrhea associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol. *For pediatric BMT studies, if specified in the protocol.* Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without	>1500mL of diarrhea/day *>15mL/kg of diarrhea/day*	Severe abdominal pain with or without ileus

grade 3 or 4 thrombocytopenia, Pain, Dehydration, Hypotension.		

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
GASTROINTESTINAL (continued).		
Duodenal ulcer (requires radiographic or endoscopic documentation)	Uncontrolled by outpatient medical management; requiring hospitalization	Perforation or bleeding, requiring emergency surgery
Gastric ulcer (requires radiographic or endoscopic documentation) Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Bleeding without perforation, uncontrolled by outpatient medical management; requiring hospitalization or surgery	Perforation or bleeding, requiring emergency surgery
Gastritis Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Uncontrolled by out-patient medical management; requiring hospitalization or surgery	Life-threatening bleeding, requiring emergency surgery
Pancreatitis Also consider Hypotension. Note: Amylase is graded in the METABOLIC/LABORATORY category.	Abdominal pain with pancreatic enzyme elevation	Complicated by shock (acute circulatory failure)

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
GASTROINTESTINAL (continued).		
Mucositis Note: Radiation-related mucositis is graded as Mucositis due to radiation.	Painless erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia
Typhlitis (inflammation of the cecum) Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Hypotension, Febrile neutropenia.	Abdominal pain, diarrhea, fever, and radiographic or biopsy documentation	Perforation, bleeding or necrosis or other life-threatening complication requiring surgical intervention (e.g., colostomy)

APPENDIX P cont'd

Grade		
Adverse Event	**3**	**4**
HEMORRHAGE		
Notes: Transfusion in this section refers to pRBC infusion. For <u>any</u> bleeding with grade 3 or 4 platelets (<50,000), <u>always</u> grade Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia. Also consider Platelets, Transfusion: pRBCs, and Transfusion: platelets in addition to grading severity by grading the site or type of bleeding. If the site or type of Hemorrhage/bleeding is listed, also use the grading that incorporates the site of bleeding: NS Hemorrhage/bleeding, Hematuria, Hematemesis, Hemoptysis, Hemorrhage/bleeding with surgery, Melena/lower GI bleeding, Petechiae/purpura (Hemorrhage/bleeding into skin), Rectal bleeding/hematochezia, Vaginal bleeding.		
Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia Also consider Platelets, Hemoglobin, Transfusion: platelets, Transfusion: pRBCs, site or type of bleeding. If the site is not listed, grade as Hemorrhage – Other (Specify site, _____). Note: This adverse event must be graded for any bleeding with grade 3 or 4 thrombocytopenia.	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia Also consider Platelets, Hemoglobin, Transfusion: platelets, Transfusion: pRBCs, Hemorrhage – Other (Specify site, _____). Note: Bleeding in the absence of grade 3 or 4 thrombocytopenia is graded here only if the specific site or type of bleeding is not listed elsewhere in the HEMORRHAGE category. Also grade as Other in the HEMORRHAGE category.	Requiring transfusion	Catastrophic bleeding requiring major non-elective intervention

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
HEMORRHAGE (continued)		
CNS hemorrhage/bleeding	Bleeding noted on CT or other scan with no clinical consequences	Hemorrhagic stroke or hemorrhagic vascular event (CVA) with neurologic signs and symptoms
Hemoptysis	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Melena/GI bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Rectal bleeding/hematochezia	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Vaginal bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Hemorrhage – Other (Specify site, _____)	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Grade		
Adverse Event	**3**	**4**
HEPATIC		
Bilirubin Bilirubin associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	>3.0 – 10.0 x ULN >6 - <15 mg/100mL	>10.0 x ULN >15 mg/100mL

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
INFECTION/FEBRILE NEUTROPENIA		
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection)	Present	Life-threatening sepsis (e.g., septic shock)
Infection/Febrile Neutropenia – Other (Specify, _____)	Severe	Life-threatening or disabling

APPENDIX P (continued)

Grade		
Adverse Event	**3**	**4**
NEUROLOGY		
Aphasia, receptive and/or expressive, is graded under Speech impairment in the NEUROLOGY category.		
CNS cerebrovascular ischemia	Transient ischemic event or attack (TIA)	Permanent event (e.g., cerebral vascular accident)
Leukoencephalopathy associated radiological findings	Severe increase in SAS; severe ventriculomegaly; near total white matter T2 hyperintensities or diffuse low attenuation (CT); focal white matter necrosis (cystic)	Severe increase in SAS; severe ventriculomegaly; diffuse low attenuation with calcification (CT); diffuse white matter necrosis (MRI)
Seizure(s)	Seizure(s) in which consciousness is altered	Seizures of any type which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)
Grade		
Adverse Event	**3**	**4**
PULMONARY		
Adult Respiratory Distress Syndrome (ARDS)	-	Present
Apnea	Present	Requiring intubation
Carbon monoxide diffusion capacity (DLCO)	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
FEV1	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
Hypoxia	Decreased O2 saturation at rest, requiring supplemental oxygen	Decreased O2 saturation, requiring pressure support (CPAP) or assisted ventilation

APPENDIX P (Continued)

Grade		
Adverse Event	**3**	**4**
RENAL/GENITOURINARY		
Creatinine *Note: Adjust to age-appropriate levels for pediatric patients.*	>3.0- 6.0 x ULN	>6.0 x ULN
Renal failure	Requiring dialysis, but reversible	Requiring dialysis and irreversible
SECONDARY MALIGNANCY		
Secondary Malignancy – Other (Specify type, _____) excludes metastasis from initial primary	-	Present

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.

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
STAGING OF CLL

Binet staging

Binet stage	Lymph node areas	Hemoglobin <11 g/dl	Platelet $< 100 \times 10^9$/L	Survival (years)
A	< 3	No	No	12
B	3 or >	No	No	7
C	±	Yes (or low platelet)	Yes (or low hemoglobin)	2

Modified Rai staging

Rai stage	Risk category	Lymphocytosis	Lymph node enlargement	Spleen/liver enlargement	Hemoglobin < 11 g/dl	Platelet $<100\times10^9$/L	Survival (years)
0	Low	Yes	No	No	No	No	> 10
I	Intermediate	Yes	Yes	No	No	No	> 7
II	Intermediate	Yes	±	±	No	No	> 7
III	High	Yes	±	±	Yes	No	1.5
IV	High	Yes	±	±	±	Yes	1.5

Appendix S

Weight / Adjusted Body Weight for Drug Dosing

Weight for Drug Dosing

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