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SEATTLE CHILDREN'S
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1. Title of protocol: A Phase II Study of Optimally Dosed Clofarabine in Combination with Low-Dose TBI to Decrease Relapse Rates after Related or Unrelated Donor Hematopoietic Cell Transplantation in Patients with AML

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2. INTRODUCTION

The incidence of acute myeloid leukemia (AML) increases nearly exponentially as a function of age beyond the third decade of life [1]. Patients diagnosed with de novo AML who are older than 55 years and receive intensive induction chemotherapy experience complete remission (CR) rates of approximately 50%, median survivals of 6-12 months, and 5 year survivals of 5-10%. Outcomes are poor even for patients who achieve CR and receive intensive consolidation therapy; the median survivals are approximately 10-12 months [2-6]. The feasibility of allogeneic hematopoietic cell transplantation (HCT) to improve relapse-free survival in patients with AML in this older age group has traditionally been limited by high rates of transplant-related mortality (TRM) due to toxicities from typical, intense conditioning regimens[7,8]. More recently, we have explored a minimally toxic nonmyeloablative preparative regimen consisting of fludarabine (90 mg/m^2) and low-dose total body irradiation (TBI; 2 Gy), which permits allogeneic engraftment and depends on graft-vs.-leukemia (GVL) effects for eliminating residual leukemic cells [9-12]. We observed long-term disease-free survival in a significant proportion of older patients with AML given either related or unrelated donor HCT [13,14], treated on Fred Hutchinson Cancer Research Center Protocols 1225, 1406, 1463, 1533, 1591, 1596, 1641, 1654, 1668, 1732, 1813, 1898, 1938 and 1959. However, a major cause of failure of this approach has been leukemia relapse, seen in 43% of patients at 5 years after HCT[14]. Here, we propose to improve outcomes for patients with AML by substituting clofarabine for fludarabine in the conditioning regimen under the hypothesis that clofarabine, a new generation purine nucleoside analogue, has comparable immunosuppressive but better anti-leukemic activities than fludarabine. As a result, we anticipate that clofarabine will reduce the leukemic cell burden before transplantation without compromising the favorable toxicity profile of this regimen. Reducing the tumor burden is likely to increase the success of GVL effects in eliminating the last leukemic cells and, thereby, minimizing the relapse risk. Patients older than 55 years with AML in morphologic leukemia-free state will be eligible for this study. Younger patients with comorbid conditions excluding them from conventional high-risk conditioning regimens will also be eligible. The efficacy part of the study will be preceded by a dose finding part, aimed at determining the optimal clofarabine dose. Monitoring for minimal residual disease (MRD) after HCT will be rigorous, using immunophenotypic, cytogenetic and molecular methods. Finally, clofarabine might be less immunosuppressive than fludarabine, and graft rejection beyond the historical rate of 5% might become an issue. Therefore, graft rejection will be monitored and the TBI dose increased by 1 Gy if more than 5% graft rejection is seen.

3. BACKGROUND

Epidemiology and Prognosis

The prevalence of AML increases with age, with a median patient age of 67 years at diagnosis, according to Surveillance, Epidemiology and End Result (SEER) statistics. Approximately 35-80% of adult patients with de novo AML achieve complete remissions when treated with induction therapy [15,16]. These remissions are rarely durable and additional therapy is needed for long-term relapse-free survival. Based on a study from the Cancer and Leukemia Group B, multiple cycles of high-dose cytarabine have

become the standard consolidation therapy for patients with favorable and intermediate cytogenetic risks under the age of 60 years [17]. The biology of AML in the elderly appears to differ from that of younger patients, with an increased incidence of antecedent dysplasia, poor prognosis karyotypes, and multidrug resistant glycoprotein MDR1 [18-21]. Furthermore, patients over the age of 60 years and those with comorbid conditions are usually treated with less intense regimens due to their inability to tolerate multiple cycles of high-dose chemotherapy, resulting in low, approximately 10-15% long term survival rates [5,22,22]. A recent retrospective analysis including 2,444 patients with AML, 60 years or older, treated on several SWOG, ECOG and M.D. Anderson protocols between 1976 and 2004 reported a 5-year overall survival rate of about 5%[23]. Allogeneic HCT using conventional high-dose conditioning regimens represents a post-remission therapy option with curative potential; however, these regimens are accompanied by prohibitively high regimen-related morbidity and mortality in older patients or those with comorbid conditions.

Nonmyeloablative HCT for patients with AML

The development of nonmyeloablative and reduced-intensity conditioning regimens has enabled older and medically infirm patients with myeloid malignancies to undergo allogeneic HCT. A number of studies of reduced-intensity conditioning followed by allogeneic HCT from HLA-identical siblings or HLA-matched unrelated donors have been published [24-28].

Among the largest studies is that from the FHCRC/Seattle Consortium, which has been recently updated [14]. The study included 274 patients (median age: 60 years) with de novo or secondary AML who underwent allogeneic HCT from related (n=118) or unrelated donors (n=156) after conditioning with 2 Gy total body irradiation (TBI) with or without fludarabine (90 mg/m^2) on FHCRC protocols. A calcineurin inhibitor and MMF were used for postgrafting immunosuppression. With a median follow-up of 38 months in surviving patients, the estimated 5-year overall survival, relapse/progression and non-relapse mortality (NRM) rates were 33%, 42% and 26%, respectively (**Figure 1**). Patients in first and second complete remission had better survivals than patients with more advanced disease (37% and 34% vs. 18%, respectively). Patients with HLA-matched related or unrelated donors had similar survivals. Advanced disease stage and unfavorable cytogenetics were associated with increased relapse and mortality. Chronic GVHD was associated with lower relapse risk.

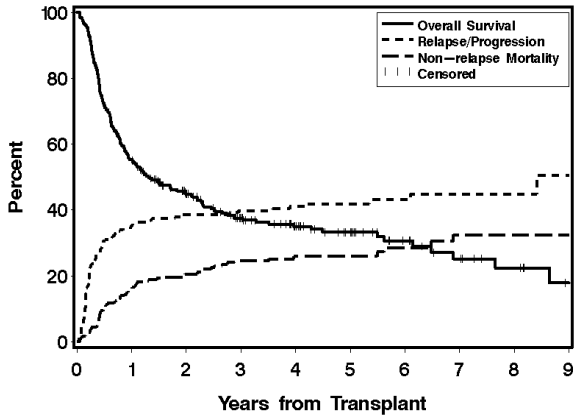


Figure 1. Overall Survival, Relapse/Progression and Non-relapse Mortality rates in patients with AML (n=274) undergoing non-myeloablative allogeneic HCT.

Despite an encouraging number of long-term leukemia-free survivors, AML relapse remained the major cause of treatment failure after HCT. Most relapses occurred early after HCT; the median time to relapse was 84 days. The 6-month and 1-year relapse rates were 31% and 35%, respectively. Relapses occurring beyond 2 years were rare.

Variable	HR	P
Stage: CR1	1.0	< 0.0001
CR2	2.51	
Not CR1/2	5.89	
AML Etiology: De Novo	1.0	0.97
Secondary	0.99	
Minimal Residual Disease: No	1.0	0.83
Yes	1.07	
Cytogenetic Risk: Favorable/Intermediate	1.0	0.001
Unfavorable	2.71	
Undetermined Significance	1.18	
CBC Recovery at HCT: Yes	1.0	0.06
No	1.55	
Time between Dx and HCT: < 6 mo	1.0	< 0.0001
6 - 18 mo	0.81	
> 18 mo	0.15	

Table 1. Risk of Relapse/Progression. Multivariate analysis, n=226.
Not significant: age, donor, HCT-CI, number of pre-HCT high-dose chemotherapy cycles

The finding of early relapse implies that regrowth of residual leukemia after HCT outpaces GVL effects, which may take months to develop. Therefore, a strategy that retards the regrowth of leukemia after HCT may provide time for potent GVL effects to develop, and, thereby, lower the relapse rate and improve survival. To this end, we propose to intensify the anti-leukemic efficacy of the current conditioning regimen by substituting the anti-leukemic drug clofarabine for fludarabine.

Development of Relapse Risk Score

In an effort to discriminate patients at high and low risk for relapse/progression within this cohort, Cox regression was used to perform univariate and multivariate analyses of risk factors (**Table 1**), treating non-relapse mortality as competing risk. Leukemia stage, cytogenetic risk status and time between diagnosis and HCT had statistically significant impact on the risk of relapse/progression.

In order to stratify patients with respect to risk of relapse, we developed a simple risk score that summarizes the contribution of multiple risk factors. From a multivariate Cox regression model (excluding patients not in remission at the time of HCT), we assigned weights based on the relative magnitude of the log hazard ratios associated with the principal risk factors. From a starting score of 0, points are added or subtracted based on the following risk factors: 2nd CR, 3rd or later CR, unfavorable cytogenetics, absence of pre-HCT peripheral blood cell count recovery and time from diagnosis to HCT > 18 months (**Table 2**). Patients were then stratified according to whether the total risk score was $\leq$0 (low risk) or >0 (high risk), providing a clear separation of relapse rates (**Figure 2A**) and overall survival (**Figure 2B**). Although this risk scoring system has not yet been prospectively validated, we believe it is adequate for risk stratification in the proposed protocol. While all patients enrolled in this study will receive the same treatment regimen, the two risk groups will be analyzed separately, given the differences in the expected relapse rates.

Variable	Score
Unfavorable Cytogenetics	+1
CR2	+1
≥ CR3	+2
Lack of pre-HCT CBC Recovery	+0.5
> 18 months between diagnosis and HCT	-2

Relapse Risk Score ≤ 0: LOW RISK
Relapse Risk Score > 0: HIGH RISK

Table 2. Relapse Risk Score

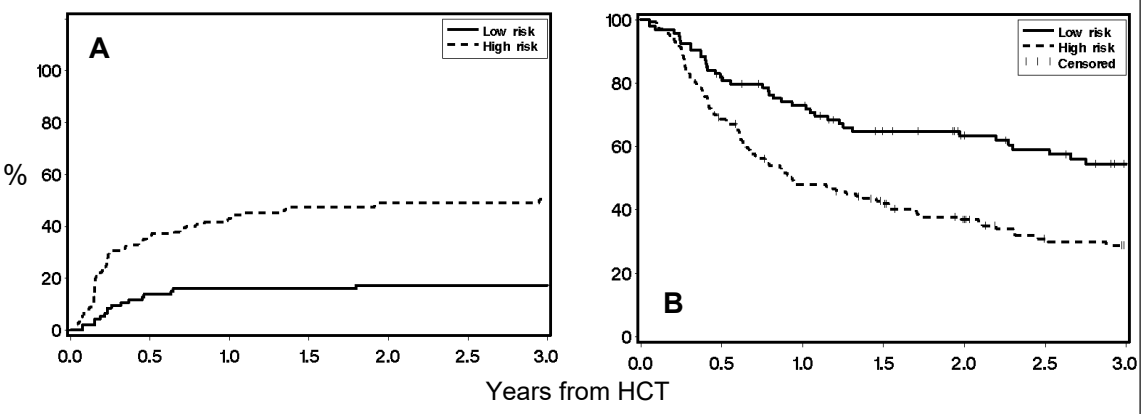


Figure 2. Relapse/Progression (A) and Overall Survival (B) rates stratified by Relapse Risk Score

Clofarabine to Reduce Relapse Risk

Clofarabine (2-chloro-9-[deoxy-2'-fluoro-β-D-arabinofuranosyl] adenine; Cl-F-ara-A; CAFdA) is a rationally designed, second generation purine nucleoside analogue. It was designed as a hybrid molecule to both overcome the limitations and incorporate the best qualities of both fludarabine (F-ara-A) and cladribine (2-CdA, CdA). It was approved in December 2004 by the US Food and Drug Administration for the treatment of pediatric patients with relapsed or refractory acute lymphoblastic leukemia after at least 2 prior regimens, based on the induction of complete responses.

In a phase I study involving pediatric patients with refractory and relapsed leukemia, six clofarabine dose levels between 11.25 and 70 mg/m^2/day were studied; the maximum tolerated dose was found to be 52 mg/m^2/day for 5 days, which resulted in complete and sustained inhibition of DNA synthesis [29]. In addition, clofarabine demonstrated efficacy in early phase studies in older or relapsed patients with AML. In phase 2 clinical trials, treatment with single agent clofarabine [30] or in combination with cytarabine [31 32], demonstrated efficacy as induction therapy as well as salvage therapy in adult patients with AML resulting in overall response rates ranging from 41% to 60%. Safety and efficacy have not been established in adults (greater than 21 years of age) or patients aged 65 years and older (Prod Info Clolar(R) iv injection, 2008). Furthermore, several groups of investigators described incorporation of clofarabine into conditioning regimens for allogeneic HCT, in combination with a traditionally used alkylating agent such as busulfan, melphalan or thiotepa [33-38,38,39]. These single institution studies, albeit small, showed, that conditioning regimens incorporating clofarabine in various combinations (mostly in combination with an alkylating agent), followed by allogeneic HCT were well tolerated and safe. The replacement of fludarabine with clofarabine in busulfan-containing preparative regimens did not seem to impact engraftment.

The anti-tumor activity of clofarabine involves three major mechanisms: inhibition of DNA synthesis (through inhibition of DNA polymerase α), inhibition of ribonucleotide reductase (RnR) and direct induction of apoptosis [40,41]. With respect to inhibition of RnR, clofarabine is superior to fludarabine [42], while in inhibiting DNA polymerase α,

clofarabine and fludarabine are similar and both are superior to cladribine [40]. In addition, unlike fludarabine, clofarabine can directly induce apoptosis by disrupting the integrity of mitochondria and thereby releasing cytochrome c and apoptosis-inducing factor [43], which is postulated to be a factor in the potent cytotoxic effects of both clofarabine and cladribine toward nondividing lymphocytes. Furthermore, studies have shown that inhibiting either RnR or DNA polymerase activity enhances tumor cell radiosensitivity by preventing the repair of DNA damage and the ability to overcome replication blockage[44,45]. Indeed, clofarabine has been shown to act as a powerful radiosensitizer both *in vitro* and *in vivo* by interfering with the DNA damage response, an effect, which was comparable to the radiosensitizing effects of 5-FU and gemcitabine[46]. These properties may be particularly effective not only in enhancing specific anti-leukemia effects, but also, promoting allograft engraftment by suppressing host T-lymphocytes in the non-myeloablative HCT setting.

We therefore hypothesize that by substituting clofarabine for fludarabine in the nonmyeloablative preparative regimen, recurrence rates of AML will be reduced without compromising engraftment or the favorable toxicity profile of this transplant approach.

4. PROPOSAL

The current multi-institutional protocol's primary objective is to: Improve the 6 month relapse rate of patients with AML who are either $\geq$ 55 years of age undergoing related or unrelated HCT following nonmyeloablative conditioning while maintaining historic rates of NRM and engraftment, or patients <55 years of age and are considered to be at high risk for serious toxicities associated with a conventional, high-dose preparative regimen. The study aim is to reduce the relapse rate to $\leq$ 20% in high-risk patients and to $\leq$ 5 % in low-risk patients (low risk group terminated August 2014 – see statistical section). We plan to achieve this goal by substituting clofarabine, a new generation purine nucleoside analogue with both immunosuppressive and anti-leukemic activity for fludarabine in the standard low-dose TBI (2, 3, or 4 Gy) regimen. The study will be conducted in 2 parts; first, a dose-finding part to determine the optimal dose of clofarabine followed by an extended accrual at the "optimal" dose. During Part 1, only patients with a relapse risk score > 0 ("high risk", as described above, in Section 2. Background) will be enrolled. We will start by administering clofarabine daily on days -6 to -2 at 30 mg/m^2/day (total dose: 150 mg/m^2). If there is no dose limiting toxicity (DLT; see definition below) when the 3rd patient reaches day 14 after HCT (21 days after initiation of clofarabine), the next three patients will be treated with clofarabine at 40 mg/m^2/day (total dose: 200 mg/m^2), with the goal to treat the final three patients at 50 mg/m^2/day (total dose: 250 mg/m^2). Once the optimal dose of clofarabine is determined, 33 additional high risk patients and 30 additional low risk patients (low risk group terminated August 2014 – see statistical section) will be treated with clofarabine at the maximum tolerated dose determined in Part 1. Participation in clofarabine pharmacokinetic studies will be offered to patients with actual body weight > 15 kg treated at the Fred Hutchinson Cancer Research Center, at all dose levels (pharmacokinetic samples discontinued January 2017). A stopping rule for graft rejection will be based on the outcomes in patients. Should that stopping rule be triggered, the dose of TBI will be increased by 1 Gy. In addition, a

stopping rule is in place for NRM, defined as any death occurring in the absence of relapse. If there exists reasonable evidence that the true rate of 100 day NRM exceeds 5%, the study will be referred to the DSMB for review to determine whether modifications are warranted or whether it needs to be terminated due to lack of safety

5. OBJECTIVES

Primary Objectives

Part 1: To determine the maximum tolerated dose of clofarabine in combination with 2, 3, or 4 Gy TBI in preparation for HCT from HLA-identical related and HLA-matched unrelated donors in patients with AML.

Part 2: To determine the efficacy of the maximum tolerated dose of clofarabine combined with 2, 3, or 4 Gy TBI in reducing the 6 month relapse rate in patients with AML compared to our historical experience with fludarabine and 2 Gy TBI. A satisfactory improvement will be considered 6 month relapse rate declines from 35% to 20% among high-risk (objective for low risk group terminated August 2014 – see statistical section)

Secondary Objectives

1. Leukemia-free and overall survivals

2. NRM of < 5% at 100 days

3. Engraftment rate of ≥ 95%

4. Prognostic significance of cytogenetics and genetic markers not detected by traditional karyotype analysis, with special respect to tyrosine kinase receptor mutations (such as FLT3), RAS- and nucleophosmin gene mutations along with C/EBP mutations

5. Rigorous monitoring for minimal residual/recurring disease by standard morphologic, flow cytometric, and molecular techniques in order to facilitate early intervention.

6. To evaluate the pharmacokinetics of clofarabine (pharmacokinetic samples discontinued January 2017).

6. PATIENT SELECTION

Inclusions
1. Patients age ≥ 55 years with AML OR patients age < 55 years with AML, who also through pre-existing medical conditions or prior therapy are considered to be at high risk for serious toxicities associated with a conventional, high-dose preparative regimen. Patients must be older than 1 year of age.

2. Patients must be in morphologic leukemia-free state (marrow blasts < 5%) without evidence of extramedullary disease within 21 days of HCT.

3. Only patients with Relapse Risk Score > 0 ("high risk") will be enrolled during Part 1 (see Relapse Risk Score system in section 3. Background). Patients with all Relapse Risk Scores will be enrolled during Part 2 (low risk group terminated August 2014 – see statistical section).

4. HLA-identical related or HLA-matched unrelated donor available.

5. A signed informed consent form or minor assent form

Exclusions

1. AML FAB M3 in CR1.

2. Active AML involvement of the central nervous system (CNS) with disease refractory to intrathecal chemotherapy (for LP requirement and intrathecal treatment guidelines, see **Appendix N**).

3. Presence of circulating leukemic blasts in the peripheral blood detected by standard morphology

4. Patients who are HIV+ (HIV+ patients registered at FHCRC should be offered treatment on Protocol 1410).

5. Fertile men and women unwilling to use contraceptive techniques during and for 12 months following treatment.

6. Organ dysfunction:
    a. Cardiac: left ventricular ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of < 26%). Ejection fraction is required if age > 50 years or there is a history of anthracycline exposure or history of cardiac disease. Patients with a shortening fraction < 26% may be enrolled if approved by a cardiologist
    b. Pulmonary:
        (i) DLCO<40% (corrected), TLC < 40%, FEV1 < 40% and/or receiving supplementary continuous oxygen.
        (ii) The FHCRC PI of the study must approve enrollment of all patients with pulmonary nodules.
    c. Liver Function Abnormalities: Patients with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, and the degree of portal hypertension. Patients will be excluded if they are found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices,

hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bridging fibrosis, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3mg/dL, or symptomatic biliary disease.

d. Renal: Serum creatinine should be within normal limits as specified by institutional guidelines. For patients with serum creatinine > upper limit of normal, a 24-hour creatinine clearance will be performed and should be equal to or more than the lower limit of normal

7. Karnofsky score < 60 (see **Appendix B**) or Lansky Score < 50 (see **Appendix C**)

8. Patients with poorly controlled hypertension and on multiple antihypertensives.

9. Females who are pregnant or breastfeeding.

10. Patients with active non-hematologic malignancies (except non-melanoma skin cancers) or those with non-hematologic malignancies (except non-melanoma skin cancers) who have been rendered with no evidence of disease, but have a greater than 20% chance of having disease recurrence within five years.

This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy.

11. 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.

12. Fungal infections with radiological progression after receipt of amphotericin B or active triazole for greater than 1 month.

13. Patients with active bacterial or fungal infections unresponsive to medical therapy.

7. DONOR SELECTION

Inclusions

1. FHCRC matching allowed will be Grade 1.0 to 2.1 (**Appendix O**): Unrelated donors who are prospectively:

a. Matched for HLA-A, B, C, DRB1 and DQB1 by high resolution typing;

b. ***Only a single allele disparity*** will be allowed for HLA-A, B, or C as defined by high resolution typing (see **Appendix O** for other donor selection details).

2. A positive anti-donor cytotoxic crossmatch is an absolute donor exclusion. 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.

3. Patient and donor pairs homozygous at a mismatched allele are considered a two-allele mismatch, i.e., the patient is A*0101 and the donor is A*0102, and this type of mismatch is not allowed.

4. Peripheral blood stem cells (PBSC) only will be permitted as a HSC source on this protocol.

Exclusions
1. Marrow donors.

2. Donors who are HIV-positive and/or medical conditions that would result in increased risk to the donor G-CSF mobilization and PBSC collections.

3. Identical twin

4. Any contraindication to the administration of subcutaneous G-CSF at a dose of 16 mg/kg/day for 5 consecutive days

5. Serious medical or psychological illness

6. Pregnant or lactating females

7. Prior malignancy within the preceding 5 years, with the exception of non-melanoma skin cancers

8. Children < 12 years old.

8. INFORMED CONSENT

A conference will be held with the patient and family to discuss this study and alternative treatments available for the underlying disease. A separate conference will be held for the donor. The conference will be conducted by the outpatient-attending physician. All potential risks associated with the use of clofarabine, low dose TBI, immunosuppressive drugs, HCT, GVHD, infections, rejection, disease progression/recurrence, risk of infertility and DLI should be discussed as objectively as

possible. Specifically, the advantages and risks of this approach in comparison to non transplant strategies should be discussed. 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.

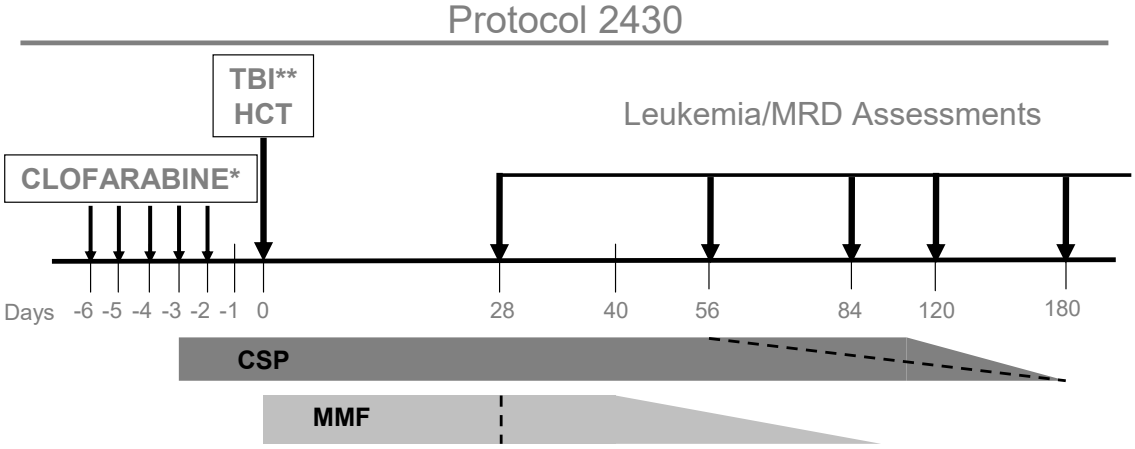
9. PROTOCOL REGISTRATION

A. FHCRC patients: Eligible patients will be identified by the Clinical Coordinators Office 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/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 Brenda Sandmaier, MD (206-667-4961).

10. PLAN OF TREATMENT

A. Outline of Treatment Plan (Figure 3. Also, please see **Appendix T** for Plan of Treatment in a Flow Chart Format)



***Clofarabine Dose Escalation**
(20 mg/m^2 x 5, total dose = 100 mg/m^2)
Dose Level 1: 30 mg/m^2 x 5, total dose = 150 mg/m^2
Dose Level 2: 40 mg/m^2 x 5, total dose = 200 mg/m^2
Dose Level 3: 50 mg/m^2 x 5, total dose = 250 mg/m^2

****TBI Dose Escalation:**
ONLY for engraftment failure
2 Gy (starting dose)
3 Gy
4 Gy

Immunosuppression
CSP: - Related Donors: 5 mg/kg PO q12hrs days -3 to +56, then taper to day +180
- Unrelated Donors: 5 mg/kg PO q12hrs days -3 to +100, then taper to day +180
MMF: - Related Donors: 15 mg/kg PO q12hrs days 0 to +28, then stop
- Unrelated Donors: 15 mg/kg PO q8hrs days 0 to +40, then taper to day +96

Figure 3. Protocol Treatment Schema

B. HCT: The hematopoietic graft will be unmodified, G-CSF-mobilized PBSC, collected as per NMDP standard, see additional details in **section G** (Collection and Infusion of Donor PBSC). Two 12-liter leukapheresis will be on consecutive days, day –1 and day 0; cells will be infused together on day 0 following TBI. Refer to institutional practice guidelines for methods of infusion. If the CD34+ cell dose is <5.0 x 10^6/kg after the second collection, a third day collection should be added, and extra dose of G-CSF should be given to the donor before the final collection.

C. Cytoreduction: Given the high risk of relapse for patients with AML allografted in relapse and the poor response to DLI for morphologically relapsed AML after allogeneic transplant, morphologic leukemia-free state (marrow blasts < 5%, the absence of circulating blasts) must be achieved prior to enrollment. The referring physician may administer therapy prior to the patient arriving at the transplant center. No high-dose 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. The attending physician will determine the need for further cytoreductive therapy.

Tyrosine Kinase Inhibitors must be discontinued on day -7 and not be resumed post HCT.

D. CNS leukemia: Patients must be evaluated for CNS leukemia with lumbar puncture, cytospin and flow cytometry of the cerebrospinal fluid prior to enrollment. Patients with a history of treated CNS leukemia are eligible if there is no evidence CNS leukemia with lumbar puncture, cytospin and flow cytometry of the cerebrospinal fluid prior to enrollment. Patients with a history of treated CNS leukemia will receive two doses of prophylactic intrathecal (IT) chemotherapy (either methotrexate or ara-c) prior to conditioning and 6 doses of IT chemotherapy following transplant starting on day +32.

E. Conditioning Regimen (refer to Figure 3):
The conditioning regimen consists of clofarabine and TBI.

Clofarabine will be administered as a 2 hour intravenous infusion once daily for 5 days, on days -6, -5, -4, -3 and -2.

Part 1

During Part 1, only patients with a relapse risk score > 0 ("high risk", as described above, in Section 3. Background) will be enrolled. The study will initially treat three patients with clofarabine at 30 mg/m^2/day (based on actual bodyweight). If there is no evidence of DLT (see **DLT** below) when the 3rd patient has reached post HCT day 14, the next group of three patients will be treated with clofarabine at 40 mg/m^2/day (based on actual bodyweight), with the goal to treat the final group of three patients at 50 mg/m^2/day(based on actual bodyweight)..

If >1 patient experiences DLTs at a given clofarabine dosage, this will define excessive toxicity, and the next lower dose level will be used for Part 2. If only one patient experiences DLTs, then 3 additional patients will be treated at that clofarabine dosage. If there are no DLTs present in these additional three patients, dose escalation will continue.

If excessive toxicity is seen at the starting dose level of 30 mg/m^2/day, then de-escalation to 20 mg/m^2/day will occur.

Clofarabine Related Dose-limiting toxicity (DLT) is defined as grade 4 toxicity of the

a) lungs (not including previous infections),
b) heart,
c) liver, not resolving within 48 hours
d) kidneys, not resolving within 48 hours
e) gastrointestinal tract
f) central nervous system

as defined by NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE), occurring within 21 days of initiation of clofarabine administration (post HCT day 14).

Patients that experience DLTs will have further clofarabine doses held and proceed to TBI as previously scheduled.

Part 2
Once the optimal dose of clofarabine is determined in Part 1, accrual will be extended to patients with relapse risk score of ≤ 0 ("low risk") (low risk group terminated August 2014 – see statistical section) and > 0 ("high risk", see description of relapse risk score in Section 2. Background). All additional patients will receive the same treatment regimen using clofarabine at that dose, but patients at high- and low risk for relapse will be analyzed separately (low risk group terminated August 2014 – see statistical section). Patients enrolled from Part 1 at the same dose used in Part 2 will be included in this analysis. We plan to treat 36 patients in the high-risk group and 30 patients in the low-risk group (low risk group terminated August 2014 – see statistical section) at this dose of clofarabine (see section 15. Statistical Considerations and Termination of Study for additional details).

Clofarabine Pharmacokinetics (discontinued January 2017)
Participation in clofarabine pharmacokinetics studies will be optional and will be offered to patients with actual body weight > 15 kg and treated at the FHCRC at the time of enrollment.

Following the infusion of the first clofarabine dose, 2-3 ml of blood will be drawn from the Hickman venous access line at each of the following time points relative to the START of the 2-hour infusion: end of clofarabine infusion (2 hr), 3 hr, 4 hr, 5 hr and 6 hr. For the 2nd, 3rd, 4th and 5th doses of clofarabine, 2-3 ml of blood will be obtained before EACH dose.. This constitutes a total of 9 pharmacokinetic samples, which will be used to obtain data on the levels of clofarabine.

TBI of 2 Gy at 6-15 cGy/min at the patient midplane from a linear accelerator will be administered on day 0, followed by PBSC infusion. Regardless of the actual time of TBI administration on Day 0, immunosuppression should be given per schedule and prior to the infusion of PBSC.

The dose of TBI will be increased by 1 Gy ONLY if there is reasonable evidence that the rate of graft rejection exceeds the historical rate of 5% (see section on "Statistical Considerations and Termination of Study" for additional details).
In this event, depending on the current toxicity experience, the dose of clofarabine will be left at the same level or reduced one dose level. In either case, the next 6 patients will be monitored for DLT. If the dose level of clofarabine is reduced, then it will be restored to the previous level if no more than one DLT is seen in the next 6 patients. If the dose level is not reduced, and more than one DLT is seen in the next 6 patients, then the dose of clofarabine will be reduced one level. After dose adjustment the next 6 patients will be monitored for DLT in the same manner.

F. Immunosuppression (refer to Figure 3.):

Day –3, patients with related donors: Commence CSP at 5.0mg/kg PO q12hrs, continue to day +56 and then taper to day +180. CSP should be routinely taken at 9:00 a.m. and 9:00 p.m.

Day –3, patients with unrelated donors: Commence CSP at 5.0mg/kg PO q12hrs, continue to day +100 and then taper to day +180. CSP should be routinely taken at 9:00 a.m. and 9:00 p.m.

Day 0, patients with related donors: **After** HCT on day 0, MMF will be given at 15mg/kg PO at 4-6 hours after G-PBMC infusion is complete, then to be given at 15mg/kg PO q12hrs until day +28 included, then, in the absence of GVHD, stopped without tapering.

Day 0, patients with unrelated donors: **After** HCT on day 0, MMF will be given at 15mg/kg PO at 4-6 hours after G-PBMC infusion is complete, then to be given at 15mg/kg PO q8hrs until day +40 included, and then taper to day +96.

CSP

1. Starting Dose in Adults: CSP (Neoral is preferred) is given orally at 5.0 mg/kg q12hrs PO (based on adjusted body weight) from day –3 until day +56 to patients with related donors and to day +100 to patients with unrelated donors. Dose should be adjusted to maintain a high therapeutic CSP level as discussed below. 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. The conversion from oral CSP (Neoral) to intravenous cyclosporine = oral cyclosporine dose divided by 2.5 equals IV dose. Use CSP levels to further adjust the dose. The formulation of CSP can be changed to Sandimmune if nausea and vomiting are persistent.

2. Pediatric Dose: Due to the variable and increased metabolism in children, CSP will be started intravenously at the following doses at day –3 to be adjusted to maintain a therapeutic level as specified below. CSP trough level should be obtained on day 0 and CSP dose adjusted to ensure therapeutic levels. The patient may be changed to oral CSP after HCT when he or she is able to take oral medications. Dose should be adjusted to maintain high therapeutic levels as discussed below. If nausea or vomiting occurs at any time during CSP treatment, CSP should be administered intravenously at the dose that was used to obtain a therapeutic level. The conversion from oral CSP (Neoral) to intravenous cyclosporine = oral cyclosporine dose divided by 2.5 equals the IV dose. Use CSP levels to further adjust the dose.
   - Age $\leq$6 years old: 1.6mg/kg IV q8hrs
   - Age >6 years old: 2.0mg/kg po or IV q12hrs. Administration route (po or IV) will be left to the discretion of Pediatric Attending.

3. In the absence of GVHD, for related donor graft recipients: CSP is to be tapered from day +56 and discontinued on day + 180; for unrelated donor graft recipients: CSP is to be tapered from day +100 and discontinued on day +180. The referring physician, who will receive explicit instructions and guidelines for detecting and managing GVHD, will manage this. Modifications of the taper schedule may be indicated if significant disease progression occurs early post-transplant

4. 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.

5. Dose Adjustments: CSP, whole blood "trough" levels (i.e. 11-12 hours from the prior dose) will be evaluated on day “0” of transplant and adjusted if necessary to maintain blood levels of 400 ng/ml (upper end of the therapeutic range of the FHCRC CSP assay). After day + 28, typical serum CSP transplant levels between 120 and 360 will be targeted. The rational for requiring initial high CSP doses is extrapolated from the preclinical nonmyeloablative canine studies, which used an equivalent dose to establish an allograft. Dose reductions should only be made if CSP toxicity (such as renal insufficiency) is present, or levels exceed values provided in Table 3. in the absence of toxicity. Dose reductions for high levels with or without toxicity should be conservative e.g. 25%, to avoid inadequate immune suppression. A decrease of the GFR by $\geq$50% or increase of creatinine of 2x baseline values attributable to CSP therapy will be justification for a 25% decrease in CSP dose. However, therapeutic levels of CSP should be maintained.

Table 3: CSP Dose Adjustment by Levels

	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 ng/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 ng/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

6. CSP Monitoring: Further CSP determinations should be performed on a twice weekly basis for the first month and then weekly until day +100 unless high levels are detected (see Table 3), or toxicity is suspected in which case more frequent monitoring will be performed as clinically indicated. Routine monitoring of CSP may follow institutional guidelines beyond day +140.

7. Drugs Interactions: Drugs that may affect CSP levels are shown in **Table 4**.

Table 4: Drugs Affecting CSP Level

Decrease CSP Levels	Increase CSP Levels	Enhance Potential for Nephrotoxicity
Carbamazepine metoclopramide nafcillin octreotide phenobarbital phenytoin primidone rifampicin sulfonamides trimethoprim	azithromycin alcohol acetazolamide caspofungin clarithromycin colchicine diltiazem doxycycline erythromycin fluconazole* fluoroquinolones imipenem itraconazole* ketoconazole nicardipine nifedipine verapamil voriconazole*	Aminoglycosides Loop diuretics (furosemide) Amphotericin formulations

**Discontinuation of fluconazole, voriconazole or itraconazole may lower CSP levels, and if used as antifungal prophylaxis changes in these drugs should be avoided during the first month post-transplant.*

MMF

1. Initiating MMF Therapy: Oral administration of MMF will be given based on adjusted body weight at 15 mg/kg (45 mg/kg/day) from **the evening of day 0** (i.e. first dose to follow 4-6 hours after HCT). Patients with related donors will receive subsequent doses q12hrs, patients with unrelated donors q8hrs. Doses will be rounded to the nearest 250mg (capsules are 250mg). If there is nausea and vomiting at any time preventing the oral administration of MMF, MMF should be administered intravenously based on adjusted body weight at 15mg/kg every 12 or 8 hrs, depending on donor type, as above.

2. Tapering of MMF: Patients with related donors will receive MMF 15mg/kg q12hrs through day +28; then, in the absence of GVHD, MMF will be stopped without tapering.

   Patients with unrelated donors will be given MMF daily at 15mg/kg q8hrs through day +40 post transplant, and then in the absence of GVHD, MMF should be tapered on day +41 by 11%/week x 8 weeks and discontinued on day +96.

3. Maintaining MMF: Markedly low (<40%) donor T-cell chimerism after HCT may indicate impending graft rejection. MMF should be continued at full dose or, if MMF taper has been initiated, reinstitution of full dose MMF should occur. Consideration of graft salvage with use of pentostatin + DLI (as per protocol 1825) should be considered.

4. Guidelines for MMF dose adjustment due to drug toxicity:
If in the clinical judgment of the attending physician the observed toxicity is related to MMF administration, a dose adjustment may occur. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF).

Gastrointestinal Toxicity. Severe gastrointestinal toxicities such as gastrointestinal hemorrhage have been very rare after nonmyeloablative HCT. In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea that is considered to be due to MMF after day +28, a 20% dose reduction will be made or the drug may be given IV. If severe refractory diarrhea or overt gastrointestinal bleeding occurs, MMF may be temporarily stopped. Then MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.

Neutropenia. Based on previous experience in patients after nonmyeloablative HCT, dose adjustments are likely to occur because of hematopoietic adverse effects, in particular neutropenia. A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim, ganciclovir). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for grade IV neutropenia that persists after day +28 post-transplant. Dose reductions should be conservative (20%). After day +21, the use of G-CSF will be permitted for neutropenia. For severe toxicity related to MMF (grade IV neutropenia > 5 days refractory to G-CSF), MMF may be decreased and if neutropenia persists, MMF can be stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.

G. Collection and Infusions of Donor PBSC:

1. G-CSF Administration to Donors: All related donors will receive G-CSF 16 ug/kg/day for 5 consecutive days from day -4 to day –0 (**Table 5**). G-CSF will be administered by a subcutaneous daily injection. These doses will be administered before 10:00 a.m. each day in the Outpatient Department (OPD). 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, the plan has to be confirmed with the personnel in the apheresis room.

Timing of PBSC collection from unrelated donors 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 3) as per NMDP protocol. Donors will receive approximately 16 µg/kg of GCSF each day of mobilization. A 12 liter apheresis will be obtained on day

–1 and on day 0 for a total of 12 to 24 liters of apheresis collection that will be infused on day 0.

a. Immunophenotyping of the PBSC product will be performed by the cellular therapy laboratory and will include T-cells and their subsets, monocytes, and NK cells.

b. Collection of DLI. Donor lymphocytes will be collected from unrelated donor PBSC products prior to transplant for potential future use of DLI on other protocol or treatment plans. A portion of the PBSC product from unrelated donors will be frozen according to standard cryopreservation for DLI.

Table 5. Treatment Schema for Donor

Day	*-5*	*-4*	*-3*	*-2*	*-1*	*0*
G-CSF (~16 μg/kg)	X	X	X	X	X	X
PBSC collection					X	X

2. PBSC Collection: related donors will preferably undergo vein-to-vein collections or may receive an appropriate catheter inserted on or before day of apheresis. PBSC 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 x 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 24 liters of whole blood during the collection. The plan for PBSC collection is shown in **Table 5.**

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

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

H. 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 D.** It should be noted that two cases of recipient hemolysis have been documented in patients with minor ABO mismatch with their donor[47]. The suspected cause is donor anti-host hemagglutinin production from “passenger lymphocytes” in the donor PBSC that may expand post transplant. Therefore, patients should be monitored and treated aggressively when there is any evidence of hemolysis.

I. Post-HCT Growth Factors
Patients should in general not receive post-transplant growth factors during the first 3 weeks after HCT. Growth factors should not be given unless neutropenia develops or persists past day 21 post transplants (ANC < 500/μL).
J. Infection Prophylaxis
Recommended prophylaxis for PCP, VZV, and HSV are listed in **Appendix E** with the modification that PCP, VZV, and antifungal prophylaxis should be continued if the patient is receiving treatment for chronic GVHD. To the extent possible, use of nephrotoxic (eg, vancomycin, amphotericin B, etc) and hepatotoxic (eg, voriconazole, etc) agents is to be avoided during clofarabine administration.

During clofarabine administration and for the following 3 days (until day +1) Micafungin will be used as antifungal prophylaxis due to possible liver toxicity. Prophylactic adult dosing is 50 mg IV daily. Prophylactic pediatric dosing is: Age 2-8 years old: 2mg/kg/dose per day max of 50mg daily. Age >8-28 years old: 1mg/kg/dose per day max 50mg daily. Following that, since antifungal prophylaxis strategies are evolving, patients may receive antifungal prophylaxis as per the standard practice of the treatment institution. Standard CMV monitoring and prophylaxis should commence at the time of transplant and should continue as per standard practice. Patients who reject their graft can discontinue this infection prophylaxis.

IgG levels and IVIG Supplemental replacement will be monitored per Institutional Practice.

K. Endpoint/Treatment Failure

Disease progression will be defined as
a. Persistence or appearance of aberrant blasts on a bone marrow aspirate by flow cytometry
b. Persistence or reappearance of a previously described cytogenetic or molecular abnormality.

Relapse will be defined as the presence of >5% blasts by morphology on a marrow aspirate or the presence of circulating blasts in the peripheral blood.

Evidence of disease progression or relapse at any time point following HCT will be considered a treatment failure, and consideration will be given to withdrawal of immunosuppression, chemotherapy and/or DLI on another protocol. Such treatment will be at the discretion of the attending physician. Patients who are considered treatment failures will still be followed for outcomes.

L. Modifications of Immunosuppression for Disease Progression/Relapse and Low Donor T-cell Chimerism: This section provides guidelines for management of patients with AML progression/relapse and low donor chimerism.

1. Progression/Relapse

Evidence of AML progression/relapse 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.

In the absence of GVHD priority should be given to rapid reduction of immunosuppression, the details of which will be left to the discretion of the attending physician. One possible approach is to discontinue MMF if it's still being taken, and taper CSP over 2 weeks. Bone marrow aspirate and blood chimerism studies should be repeated when off immunosuppression after 2 weeks. An alternative approach that can be considered, especially, when there is a concern of an acute GVHD flare is the abrupt discontinuation of both CSP and MMF followed by administration of weekly low dose (10 mg/m^2) methotrexate.

DLI will not be given for progressive or relapsed disease on this protocol, and patients with relapse or progression would be eligible for other ongoing intervention protocols or treatment plans (i.e. re-induction, DLI, hypomethylating agents, TK inhibitors, etc), according to the attending physician's preference.

2. Low Donor T-Cell chimerism

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+ donor 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 evaluation. Low donor chimerism is defined as ≤ 40% CD3+ donor 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 (e.g. pentostatin + DLI as per protocol 1825) will be made based on the results of sorted T-cell studies of *peripheral blood.* For the purposes of this protocol, *graft rejection* is defined as the inability to detect or loss of detection of greater than 5% donor T-cells (CD3+) as a proportion of the total T-cell population, respectively, after nonmyeloablative HCT. Also for the purposes of this protocol, *graft failure* is defined as grade IV thrombocytopenia and neutropenia after day +28 that lasts > 2 weeks and is refractory to growth factor support. TBI dose escalation in this protocol will be based on graft rejection.

Evaluation of chimerism. Patients will have peripheral blood and whole bone marrow evaluations for chimerism at various time points through one year post transplant. If the patient has not obtained ≥ 95% donor chimerism in CD+3 by one year continue to evaluate through 5 years post transplant as clinically indicated. 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).

Decreasing Donor Chimerism and Graft Rejection. Decreasing donor chimerism after day +28 may indicate that graft rejection is occurring. DLI alone does not appear to reverse this process. The patient's case should be discussed with the FHCRC principal investigator of the study. Patients who reject their grafts will not be offered DLI on this protocol and may be eligible for a second allograft on another protocol.

11. PATIENT EVALUATIONS

A. Patient Pretransplant/Baseline Evaluation.

Work-up should include the following:

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

2. Careful physical exam with determination of Karnofsky score (**Appendix B**) or Lansky Play-Performance Score (**Appendix C**), and scoring according to the HSCT-CI Score **(Appendix Q).**

3. CBC/differential, creatinine, BUN, uric acid, Na+, K+, Cl_, Bun, creatinine, glucose, liver function tests, ABO/Rh typing, hepatitis screen, CMV, and toxoplasmosis serology, anti-HIV serology, and serum LDH

4. Obtain serum creatinine approximately 72 hours before clofarabine administration. If serum creatinine > upper limit of normal, obtain 24-hour urine collection to measure creatinine clearance.

5. Pulmonary function tests with corrected DLCO.

6. Chest x-ray (CXR), PA and lateral views.

7. MUGA scan or echocardiogram for patients > 50 years of age, or history of cardiac disease or anthracycline exposure.

8. **Evaluation and prophylaxis of CNS disease**.

Please refer to **Appendix N** for recommendations for intrathecal diagnostic evaluation and prophylaxis for specific malignant diseases. If patients undergo intrathecal diagnostic evaluation, cerebral fluid should be sent for cell count and differential, cytospin, cytology, total protein, and glucose.

9. **Immunophenotyping of the PBSC graft.**

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

Additionally, see the following table (Table 6) for AML pre-transplant evaluations.

Table 6: Pre-Transplant Evaluation

Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected within **21 days** of treatment. See Tables 7-8 for post-transplant evaluations and additional lab instructions.

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate		
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics- **see comment*	Clinical	**If abnormal pre-transplant*
FISH for clonal abnormalities - **see comment*	Clinical	**If abnormal pre-transplant*
PCR	Research – Specimen Processing	
Peripheral Blood		
Storage for chimerism analysis	Clinical	
PCR - **see comment*	Research – Specimen Processing	**Only if marrow sample is not available*

10. Retrospective Specimens: unstained bone marrow aspirate slides, core biopsy specimens (fixed in formalin) and/or peripheral blood samples and/or slides obtained at diagnosis or confirmed relapse of AML will be requested from outside institutions and will be used for cytogenetic and molecular studies (with special respect to tyrosine kinase receptor mutations, such as *FLT3, RAS*- and nucleophosmin gene mutations along with *C/EBP*α mutations). Send specimens to Specimen Processing (FHCRC E1-305), label samples “Protocol #2430”.

B. Patient Post-transplant Evaluation
See Table 7 for AML post-transplant evaluation on day +28, +56, +84, etc. This is a recommended evaluation schedule.

Additionally, include the following:
1. 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.

2. Electrolyte panel (sodium, potassium, chloride, CO2, glucose, BUN, creatinine, calcium, magnesium, phosphorus and albumin) 3 times per week and liver function tests (ALT, AST, ALK Phos, total bilirubin, direct bilirubin, total protein, albumin and LDH) 2 times per week until day +28 and then weekly.

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

4. GVHD Evaluation at Departure from Transplant Center
This evaluation should occur on the week of day + 84.

GVHD evaluation guidelines:
a. History and physical exam, attention to 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 13.F for diagnosis and treatment guidelines for acute and chronic GVHD.

Table 7: Post-Transplant Evaluation (See text for pre-transplant evaluations)
This is a recommended evaluation schedule. See Table 8 for additional lab instructions.

Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days					Years		Annual x 5 years
			28	56	84	120	180	1	1.5	
BM aspirate* If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment										
Chimerism	Clinical				x			x		
Pathology	Clinical		x	x	x	x	x	x	x	x
Flow cytometry	Clinical		x	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	*See comment
FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
PCR for *FLT3-ITD, RAS, NPM1* and *C/EBP* mutations and t(8;21), inversion 16; t(15;17)	Research - Specimen Processing, E1-305	*If molecular marker present pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
Peripheral blood										
Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x		*See comment	x		
Chimerism (CD33+)	Clinical				x					
Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x							
PCR for *FLT3-ITD, RAS, NPM1* and *C/EBP* mutations and t(8;21), inversion 16; t(15;17)	Research - Specimen Processing, E1-305	*If molecular marker present pre-transplant AND marrow sample is not available	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
GVHD evaluation	Clinical	See text for details			x					

Table 8: Additional Lab Instructions

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

Off-site providers may use local facilities for the tests.

Volumes represent desired amounts.

Specimen / Test	Type	Instructions	Lab Name	Contact Information
Bone marrow				
Chimerism	Clinical	1-3mL bone marrow in green-top tube	Clinical Immunogenetics Lab	Seattle Cancer Care Alliance (206) 288-7700
Pathology (*aspirate*)	Clinical	2mL bone marrow in EDTA/ formalin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Pathology (*biopsy*)	Clinical	1cm bone marrow in formalin **OR** mounted in paraffin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Flow Cytometry	Clinical	2mL bone marrow in green-top tube	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
Cytogenetics	Clinical	3mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
FISH	Clinical	2mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
PCR	Research	3mL bone marrow in lavender-top tube Label “protocol 2430”	Specimen Processing	FHCRC E1-305 (206) 667-4645
Peripheral blood				
Chimerism (CD3+), (CD33+) NK(CD56+)	Clinical	10mL blood in green-top tube for Flow sorting, then to CIL	UW Hematopathology Lab, routed to Clinical Immunogenetics Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
Flow Cytometry	Clinical	10mL blood in green-top tube	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
PCR	Research	7mL blood in lavender-top tube Label “Protocol 2430”	Specimen Processing	FHCRC E1-305 (206) 667-4645

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

Table 9: Retrospective Specimens

Note: Samples obtained at diagnosis of AML or first indication of relapse from outside centers

Specimen / Test	Type	Instructions	Lab Name	Contact Information
Bone marrow				
PCR	Research	3mL bone marrow in lavender-top tube AND/OR unstained slides Label “protocol 2430”	Radich Lab	FHCRC D4-385 (206) 667-2592
Peripheral blood				
PCR	Research	7mL blood in lavender-top tube AND/OR unstained slides, ONLY if marrow is not available Label “protocol 2430”	Radich Lab	FHCRC D4-385 (206) 667-2592

12. DONOR EVALUATIONS

1. Complete history and physical examination.

2. Lab tests: CBC with reticulocytes and platelet counts, serum sodium, potassium, chloride, CO2, BUN, creatinine, uric acid, LDH, calcium, magnesium, phosphate, alkaline phosphatase, AST, ALT, hepatitis screen, CMV, syphilis, HIV and HTLV I serologies and ABO Rh blood typing. If the donor has antibodies against red cell antigens of the recipient, the titers will be determined. Cytotoxic crossmatch between patient and donor will be performed (HLA Laboratory).

3. No placement of a central line is necessary for PBSC collection unless it is determined that the donor has poor venous access. If necessary, a temporary apheresis (e.g. Mahurkar) catheter will be placed at the time of leukapheresis.

4. A CBC will be checked prior to and after leukapheresis collection, and daily while on G-CSF. CBCs will be checked thereafter if clinically indicated.

5. The donor will be reevaluated with a directed history and physical examination the day after the apheresis is completed.

6. To subsequently determine the origin of relapse, heparinized blood will be sent from the donor to CIL (FHCRC: 206-288-7700) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor.

7. For females of child bearing age, serum pregnancy qualitative [PGSTAT] within 72 hours prior to initial dose of filgrastim (G-CSF). Results must be available prior to filgrastim (GCSF) dose.

13. DRUGS AND TOXICITIES

Cyclosporine, MMF and clofarabine are all commercially available. They should be stored and mixed according to manufacturer's recommendations.

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

B. Clofarabine See section **10.E Conditioning Regimen** for information about administration and dosage adjustments.

Chemical Name:
2-chloro-9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-9H-purine-6-amine

Other names:
CLOLAR, clofarabine; CAFdA; Cl-F-ara-A;
2-chloro-2′-fluoro-deoxy-9-β arabinofuranosyladenine
2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine (Cl-F-ara-A)
2-chloro-2′-arabino-fluoro-2′-deoxyadenosine
2-chloro-2′-ara-fluorodeoxyadenosine (CAFdA)
2-chloro-2′-fluorodeoxyadenosine (CAFdA)

Physical and Chemical Characteristics:
Clofarabine is a white to off-white solid with a melting point of 228°C to 230°C and a molecular weight of 303.5. The drug substance is very stable in the dry state, and aqueous solutions are stable to heat treatment. Clofarabine is freely soluble in water (1.5 mg/mL) or buffered solutions at room temperature. Clofarabine is not less than 97% pure on a dried basis by high performance liquid chromatography (HPLC) analysis. Clofarabine is formulated at a concentration of 1 mg/mL. Clofarabine is supplied in 1 vial size: a 20-mL clear, glass vial with gray stopper and blue flip off seal. The 20-mL vials contain 20 mL (20 mg) of sterile solution. The pH range of the solution is 4.5 to 7.5. The solution is clear and practically colorless, preservative free, and free from foreign matter.

Toxicity (IV Studies):
1. Adult Patients
Drug-related adverse events (AE) observed in at least 10% of adult patients treated with clofarabine in previous clinical trials include myelosuppression, nausea, vomiting, infections, fatigue, headache, diarrhea, rigors, dermatitis, anorexia, febrile neutropenia, myalgia, asthenia, petechiae, transient elevated liver enzymes, stomatitis, mucositis, pyrexia, flushing, constipation, edema, dehydration, nervousness, stomach pain, insomnia, depression, dry skin, back pain, and decreased weight.

Adverse events reported in <10% of adult patients include tumor lysis syndrome, capillary leak syndrome/SIRS, palmar plantar erythrodysesthesia, pancreatitis, seizures, irregular heartbeat, edema, pericardial effusion, multi-organ failure, and death.

2. Pediatric Patients

The most common side effects observed in previous clinical studies of pediatric patients treated with clofarabine 52 mg/m2 include vomiting NOS, nausea, febrile neutropenia, diarrhea, headache, pruritus, pyrexia, dermatitis, fatigue, rigors, abdominal pain, tachycardia, anorexia, petechiae, epistaxis, pain in limb, hypotension, anxiety, cough, constipation, erythema, mucosal inflammation, NOS, pain, flushing, edema, and hematuria.

Pediatric patients have also experienced increased liver enzymes and increased creatinine. Moderate neurological changes have been reported in some patients. Infections were reported in almost half of the patients treated with clofarabine. Other potential severe AEs include pericardial effusion, LVSD, tumor lysis syndrome, SIRS, and capillary leak syndrome.

3. Management of Capillary Leak Syndrome

In pediatric studies, during or shortly after IV clofarabine administration a few patients developed signs and symptoms consistent with capillary leak syndrome. In these heavily pretreated patients, it has been difficult to separate potential drug-related cases of capillary leak syndrome from concurrent medical conditions such as infection/sepsis, progressive disease, or other underlying problems resulting from prior antileukemic therapies.

For these reasons, during and after each dose of clofarabine investigators are to assess patients for the onset of the following signs or symptoms $\geq$ grade 2:

− Tachypnea or other evidence of respiratory distress;
− Unexplained hypotension; and/or
− Unexplained tachycardia.

If one or more of these signs or symptoms occurs during clofarabine infusion, clofarabine administration is to be interrupted or held as clinically indicated. It is recognized that the total infusion time for this clofarabine dose in this circumstance may exceed 2 hours. Thus, if the patient's condition stabilizes or improves, clofarabine administration may resume.

Pretreatment with steroids (e.g. hydrocortisone 100 mg/day or its equivalent or dexamethasone 20 mg/day as part of an anti-emetic regimen) is recommended for all subsequent doses during the remainder of clofarabine treatment.

4. Management of liver toxicity

Severe and fatal hepatotoxicity has occurred with the use of clofarabine. In clinical studies, Grade 3-4 liver enzyme elevations were observed in pediatric patients during treatment with clofarabine at the following rates: elevated aspartate aminotransferase (AST) occurred in 36% of patients; elevated alanine aminotransferase (ALT) occurred in 44% of patients. Grade 3 or 4 elevated bilirubin occurred in 13% of patients, with 2 events reported as Grade 4 hyperbilirubinemia (2%), one of which resulted in treatment discontinuation and one patient had multi-organ failure and died. Monitor hepatic function and discontinue clofarabine for Grade 3 or greater liver enzyme elevations and contact PI to discuss modification of conditioning.

C. TBI: TBI will be given in one 200, 300, or 400 cGy fraction from linear accelerator at a rate of 6 - 7 cGy/min. Dosimetry calculations are performed by the radiation therapist. At the dosage used, side effects are not expected. Nevertheless, there may be fever, alopecia, parotitis, diarrhea, reversible skin pigmentation, mucositis and late effects including cataract formation, growth retardation, pulmonary damage, carcinogenesis, and sterilization.

D. CSP: See section **10.F. Immunosuppression** 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. See standard practice manual for additional information about administration, toxicity and complications.

E. MMF: See section **10.F. Immunosuppression** for information about administration and dosage adjustments. *Mycophenolate mofetil (MMF)*: is supplied in 250mg hard gelatin capsules. Capsules may be stored at room temperature.

1. Precautions: previous clinical studies 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: administration of MMF may be associated with vomiting, diarrhea, anemia, leukocytopenia and infection. In the setting of marrow transplantation, however, several etiologic factors may contribute to these 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. 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.

F. GVHD:
1. Diagnosis: Skin involvement will be assessed by biopsy with percentage of body surface area involved recorded. GI symptoms suspicious for GVHD will be evaluated by biopsy as indicated. Acute GVHD and chronic GVHD will be graded according to established criteria (**Appendix F and G**).
2. Treatment:
Details of GVHD treatment will be left to the discretion of the attending physician. Patients may also be eligible for institutional trials of GVHD therapy.

G. Myelosuppression
Grade IV myelosuppression will be defined as a decrease in ANC to <500/uL and/or platelet count to $\leq$ 20,000/uL. If myelosuppression occurs beyond day +28, a bone

marrow aspirate and biopsy should be considered to exclude disease progression. Samples should be sent for chimerism analysis by a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or FISH studies or VNTR) of the patient and donor. Myelosuppression may occur in this patient population for a number of reasons such as direct toxic effect of drugs (clofarabine, MMF, ganciclovir etc.), rejection, relapse or after DLI. Patients who are > 21 days after HCT with an ANC of <500/uL may receive G-CSF 5µg/kg/day S.C. Thrombocytopenic patients will receive platelet transfusion as per standard care.

14. 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.

15. STATISTICAL CONSIDERATIONS AND TERMINATION OF STUDY

Part 1: dose escalation

The primary objective of Part 1 is to determine the highest dose of clofarabine that can be tolerated safely in conjunction with nonmyeloablative HCT. During Part 1, only patients with a risk score > 0 ("high risk" as described above, in Section 2. Background) will be enrolled. A standard 3+3 dose escalation scheme will be employed starting at a dose of 30 mg/m^2/day x 5 days (total dose 150 mg/m^2), as described above. If 3 patients are successfully transplanted without DLT, then escalation to the next dose level will occur. If 1 of 3 patients experiences DLT, then an additional 3 patients will be treated at the same dose, and dose escalation will only occur if no additional patient experiences DLT. A second DLT in either 3 or 6 patients will define excessive toxicity, and the next lower dose level will be used for Part 2. If the highest dose level, 50 mg/m^2/day (total dose of 250 mg/m^2) has acceptable toxicity, then that dose level will be used for Part 2. The operating characteristics of this dose escalation rule are defined in this table. If excessive toxicity is seen at the starting dose level of 30 mg/m^2/day, then de-escalation to 20 mg/m^2/day will occur.

True rate of DLT	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Probability of escalation	91%	71%	49%	31%	17%	8%	3%	1%	<1%

During Part 1, a second occurrence of graft rejection in up to 6 patients at a given clofarabine dose level will lead us to be at least 90% confident that the true rate of graft rejection exceeds the historical rate of 5%. If a single engraftment failure is observed in the first 3 patients at a given dose level, then an additional 3 patients must be enrolled at the same level without an additional engraftment failure before dose escalation can continue. If a second engraftment failure occurs, then the dose of TBI will be increased by 1 Gy. Dose escalation will restart at the current clofarabine dose, provided that

clofarabine dose escalation would have been permitted according to the criteria above; otherwise, the clofarabine dose will be decreased one level.

Part 2: efficacy analysis
The primary objective of Part 2 is to evaluate whether the substitution of clofarabine for fludarabine can improve the 6 month rate of disease relapse/progression, as defined in Section 9.K, compared to our historical experience with a Flu/TBI non-myeloablative conditioning regimen followed by HCT. The historical rates are approximately 35% at 6 months after HCT in a high risk group (risk score > 0), and approximately 15% at 6 months after HCT in a low risk group (risk score $\leq$ 0 (low risk group terminated August 2014)). The risk score for a patient is determined by the following scoring system, as described Section 2. Background: 2nd CR, +1; 3rd or later CR, +2; unfavorable cytogenetics, +1; lack of peripheral blood cell count recovery (platelet count > 100,000/μl; neutrophils > 1,000/μl), +0.5; greater than 18 months from diagnosis to transplant, -2.

All patients will receive the same treatment regimen, but the two risk groups will be analyzed separately. Patients enrolled from Part 1 at the same dose used in Part 2 will be included in this analysis.

In the high risk group, 36 patients will provide 83% power to detect a 15 percentage point improvement in the relapse/progression rate, to 20%, with a 1-sided alpha level of 0.13. A successful outcome will be defined by relapse within 6 months of HCT in 9 or fewer patients.

In the low risk group, 30 patients will provide 81% power to detect a 10 percentage point improvement in the relapse/progression rate, to 5%, with a 1-sided alpha level of 0.15. A successful outcome will be defined by relapse within 6 months of HCT in 2 or fewer patients. (low risk group terminated August 2014)

With the termination of the low risk group as of August 2014, total enrollment will be 44 patients. We expect to enter approximately 10 patients per year, so the study will take 7 years to complete enrollment.

Low risk group terminated. As of August, 2014, accrual of low risk patients in Part 2 was halted with only 2 patients accrued. All statistical considerations pertaining to the high risk group as described herein are unchanged.

Futility stopping rule (Part 2 patients, and part 1 patients at maximum tolerated dose)
. If there is reasonable evidence that the rate of relapse/progression within a risk group is not improved over the historical benchmark, then accrual to that risk group will be terminated. Reasonable evidence will be taken to mean that the lower bound of a 1-sided 80% confidence interval for the true rate of relapse/progression exceeds 35% for the high risk group or 15% for the low risk group. Operationally, this rule will be triggered by:

- High Risk Group: 6/10, 10/20, or 14/30 patients with relapse progression
- Low Risk Group: 3/10 or 5/20 patients with relapse progression (low risk group terminated August 2014)

Safety stopping rules (Part 1 Patients, part 1 patients at maximum tolerated dose, and Part 2 patients**)**

Stopping rules will be imposed for non-relapse mortality, defined as any death occurring in the absence of documented relapse or progression, and engraftment failure. The historical experience is that the NRM rate at 100 days post-HCT in this patient population is only 5%. We will halt the study for safety review by the DSMB at any time that there exists reasonable evidence that the true rate of NRM exceeds 5%. Reasonable evidence will be taken to mean that the lower bound of a 1-sided 90% confidence interval for the true rate of day 100 NRM exceeds 5%. Operationally, this rule will be evaluated at least every 10 patients as follows:

- NRM (5% threshold): 2/10, 3/20, 4/30, 5/40

Patients may continue to be enrolled pending evaluation of the day 100 endpoint, but the outcome in subsequently enrolled patients cannot override the rule if triggered in a lesser number. The operating characteristics of the rule are summarized in the table below.
Therefore, **stopping rules** will be imposed for:

- NRM > 5% at day 100

The stopping rule will be evaluated separately within each risk group. Should the rule be triggered, accrual to that risk group will be suspended, pending a review of data by our DSMB. This review will consider the possible relationship of the NRM to clofarabine, and whether a possibly increased risk of NRM could be tolerated in light of available data on the risk-benefit ratio.

Operating characteristics of the stopping rules.

True rate of NRM	Probability of triggering rule[1] (high risk/low risk[2])	Average N before stopping[1] (high risk/low risk[2])	True rate of graft rejection	Probability of triggering rule[1]	Average N before stopping[1]
5%	17% / 14%	32 / 28	5%	19%	61
10%	54% / 48%	26 / 23	10%	70%	41
15%	83% / 75%	19 / 18	15%	95%	25
20%	95% / 91%	14 / 14	20%	>99%	17

[1] based on 10,000 Monte Carlo simulations
[2] low risk group terminated August 2014

We will also continue to monitor the occurrence of graft rejection for evidence that the rate of graft rejection exceeds the historical rate of 5%. A stopping rule for graft rejection will be based on the combined risk groups, and the confidence limit will be set to 90% in order to avoid excessive risk of falsely stopping:

- Graft rejection >5%

The criteria for triggering the rule will be the same as for NRM for the first 40 patients as follows:

- Graft rejection(5% threshold): 6/50-60, 7/70

Should the stopping rule be triggered, we will increase the dose of TBI by 100 cGy. Depending on the current toxicity experience, the dose of clofarabine will be left at the same level or reduced one dose level. In either case, the next 6 patients will be monitored for DLT. If the dose level of clofarabine is reduced, then it will be restored to the previous level if no more than one DLT is seen in the next 6 patients. If the dose level is not reduced, and more than one DLT is seen in the next 6 patients, then the dose of clofarabine will be reduced one level. After dose adjustment the next 6 patients will be monitored for DLT in the same manner.

A revision to the TBI dose during Part 2 naturally could impact the evaluation of primary and secondary endpoints. A decision as to whether to extend accrual to accommodate this revision will depend on the number of patients already accrued, the preliminary results at the time of revision, and the feasibility of extended accrual. If extended accrual is appropriate and feasible, a protocol modification will be submitted for that purpose.

Additional analysis
Although the analysis of Part 2 will be stratified by risk group, comparison to historical rates is always complicated by variation in patient characteristics. Regardless of the formal outcome of the trial as defined in Part 2, we would plan to perform a more comprehensive analysis of post-transplant endpoints using our historical database as a comparator. Although obviously not a substitute for a randomized trial, it will help us to determine whether the results stratified on risk score are robust to variation in other patient characteristics.

These analyses will employ Cox regression models to compare patients in the current study to patients from our historical database for time-to-event endpoints, and will evaluate non-relapse and overall mortality, relapse and relapse-free survival, as well as acute and chronic GVHD. Although the overall sample size for the current study will be relatively modest, it will also enable us to perform preliminary validation of the scoring system for relapse risk that we have developed from our historical data.

Due to limited sample size, the evaluation of genetic markers in the secondary objectives will be largely exploratory in nature. The table below presents some

approximate power calculations indicating the magnitude of hazard ratios that could be detected using only patients in the current study.

Power to detect association of genetic markers with relapse rate

Proportion with marker	Hazard ratio	Power[1]
20%	2.5	61%
33%	2.0	47%
33%	2.5	77%
40%	2.0	51%
40%	2.5	78%

[1] assuming n=75, 2-sided alpha=0.05,
3 years of accrual and 1 year additional follow-up,
relapse rate profile similar to historical

16. DATA SAFETY AND MONITORING PLAN

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

Protocol 2430 is a multi-institutional clinical trial that is monitored by the principal investigator (PI), Dr. Brenda Sandmaier, with oversight by a Data Safety and Monitoring Board (DSMB), the Data Safety Monitoring Committee (DSMC) and the Institutional Review Board (IRB). The PI reviews outcome data with the protocol mentor for each individual patient at a minimum of 3 months after unrelated donor HCT and the updated data are presented at Mixed Chimerism Meetings (includes co-investigators). Please see **Appendix I and J** for definitions of adverse events, serious adverse events (SAE) and serious and unexpected events as well as mechanisms for reporting these events. SAEs are reported to the trial coordinator. The trial coordinators at collaborating centers or the local PIs will fax an official report of an SAE to the coordinating center (FHCRC) within ten days. The SAE report is reviewed by the PI. If the SAE meets the FHCRC criteria for expedited reporting then an official signed report is submitted to the FHCRC Institutional Review Office (IRO). All deaths, regardless of the cause, are reported to the IRB. Protocol 2430 will have a dedicated independent DSMB responsible for monitoring patient safety on this clinical trial. The DSMB will meet at approximately six month intervals for this protocol and all outcome data will be reviewed including all adverse events and SAEs reported to the coordinating center (FHCRC) along with those officially reported to the FHCRC IRO. The DSMB will confirm that the trial has or has not met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMB is submitted to the FHCRC IRB as well as the trial coordinators/local PIs of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual and the last patient treated is past day +180. Furthermore, the FHCRC also has a DSMC that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual IRB review and approval will also be required. Flow of information concerning clinical trial participants will originate with the clinicians and nurses in the clinic or referring clinicians at other institutions and is transmitted to the trial coordinator. At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes

in the medical record. This documentation is extracted by the study nurse within 140 days +/- after HCT via chart review and collection of copies of source documents and entered into a hard copy or electronic Case Report Form (CRF). The PI reviews the official CRF and primary source documents. When the CRFs are verified, they are signed by the PI.. Thus, multiple health care providers provide independent observations and participate in monitoring this trial. The PI may be a clinician for some patients entered on this trial. However, assessments are the sum total of the primary health care provider (fellow or physician assistant), floor or outpatient nurse and the PI or other attending clinician involved with the patient averting possible conflict of interest having the PI as the attending clinician for protocol patients. If determination of adverse events is controversial, co-investigators will convene on an ad hoc basis as necessary to review the primary data and render a decision. Protocol 2430 will be a multi-institutional protocol and all collaborating centers sign an agreement with the FHCRC stating that data generated from patients from the protocol will be reported accurately in a timely manner to the FHCRC. All centers have IRBs that review the protocol and who local PIs contact when an adverse event on the protocol occurs. Most of the centers have internal auditing mechanisms that assure accurate assessment of clinical outcomes. Clinical outcome data will be summarized and transmitted from collaborating centers as CRFs. When possible, primary source documents regarding patient outcomes will be collected with patients' names removed and replaced by Unique Patient Numbers (UPNs). The CRFs are generated from the collaborating centers at defined time points (100 days, 6 months, and yearly). The local PI reviews the official CRF and primary source documents. When the CRFs are verified, they are signed and data will be entered into a central database managed by the trial coordinator.

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

The adverse event reporting in this multi-institutional clinical trial will follow the FHCRC Guidelines for SAE reporting. These guidelines (attached in **Appendix I**.) detail the expedited reporting requirements, definitions of particular events. All SAEs that meet expedited reporting criteria are reported to the IRO within 10 days by the investigator, trial coordinator, or research nurse upon learning of the event. A completed SAE report form, signed by the PI, must be received by the IRO within 10 calendar days. The PI reviews all SAEs and annual reports at the time of submission. For patients being cared for at the FHCRC, health care providers communicate with the PI, trial coordinator or research nurses as events occur triggering subsequent reporting. For patients not being cared for at FHCRC the outside facilities communicate with the PI, trial coordinator, or research nurse for these reporting purposes. All other deaths and expected serious adverse events are reported to the IRB at the time of annual renewal and at the biannual mixed chimerism meeting. The PI for a study is responsible for this reporting and the IRO assures adverse event reporting on an annual basis. The PI in the annual application for grant continuation will summarize reports of toxicities. Furthermore, an additional safeguard for adverse event analysis and reporting in this protocol is provided by stopping rules that are monitored at least every 10 patients in each arm. All collaborating PIs have fulfilled all NIH requirements for training in human subjects' protection.

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

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

4. Plans for assuring data accuracy and protocol compliance

Collaborating sites will 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 will be 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.

17. TARGETED/PLANNED ENROLLMENT

Ethnic Category	Gender		
	Females	Males	Total
Hispanic or Latino	1	1	2
Not Hispanic or Latino	18	24	42
Ethnic Category Total of All Subjects	19	25	44
Racial Categories			
American Indian / Alaska Native	0	0	0
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	1	2
White	17	23	40
Racial Categories: Total of All Subjects	19	25	44

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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

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

LANSKY PLAY-PERFORMANCE SCALE
(for use with persons ages 1-6 years)

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

Appendix D

ABO INCOMPATIBILITY

Red Blood Cell - Incompatibility (Major):
Occasional patients may have antibodies directed against red blood cell antigens found on the donor's cells. These are generally ABO or Rh antigens, although incompatibility with other red cell antigens identified by donor-recipient crossmatch may occur. Although the volume of red blood cells (RBC) in most PBMC products will only be 2-5% of the product volume before infusion, the small quantity may cause a hemolytic transfusion reaction. According to the FHCRC policy it is generally acceptable to infuse a volume of about 10ml RBCs per product. If the recipient shows an anti-donor titer of $\geq$ 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 $\geq$ 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 $\geq$ 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 D (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 E

INFECTIOUS DISEASE GUIDELINES

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

Herpes Simplex and Varicella Zoster Virus Prevention and Treatment

hsv-vzv.pdf

CMV Prevention: Surveillance and Preemptive Therapy

cmvprevention.pdf

Antifungal Therapy Guidelines

Please see Section 9.J on recommendations on antifungal prophylaxis while patients are receiving clofarabine.

antifungal_therapy.pdf

Pneumonia / Pneumocystis Carinii Prophylaxis

pneumocystisjiroveci.pdf

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

antibioticprophylaxisforencapsulatedbacte

Appendix F
GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE

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

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

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

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

Appendix G

CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD)

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

A. Classification of Chronic GVHD

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

1. Chronic GVHD not requiring systemic treatment: mild abnormalities involving a single site, with platelet count >100,000 and no steroid treatment at the onset of chronic GVHD.

a) Oral abnormalities consistent with cGVHD, a positive skin or lip biopsy, and no other manifestations of cGVHD.

b) Mild liver test abnormalities (alkaline phosphatase $\leq$2 x upper limit of normal, AST or ALT $\leq$3 x upper limit of normal and total bilirubin $\leq$1.6) with positive skin or lip biopsy, and no other manifestations of cGVHD

c) Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of body surface area (BSA), dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy, and no other manifestations of cGVHD

d) Ocular sicca (Schirmer's test $\leq$5mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of cGVHD

e) Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of cGVHD

2. Chronic GVHD requiring systemic treatment: more severe abnormalities or involvement of multiple sites, or platelet count <100,000, or steroid treatment at the onset of chronic GVHD

a) Involvement of two or more organs with symptoms or signs of cGVHD, with biopsy documentation of cGVHD in any organ

b) ≥15% base line body weight loss not due to other causes, with biopsy documentation of cGVHD in any organ
c) Skin involvement more extensive than defined for clinical limited cGVHD, confirmed by biopsy
d) Scleroderma or morphea
e) Onycholysis or onychodystrophy thought to represent cGVHD, with documentation of cGVHD in any organ
f) Decreased range of motion in wrist or ankle extension due to fasciitis caused by cGVHD
g) Contractures thought to represent cGVHD
h) Oral involvement with functional impairment, refractory to topical treatment
i) Vaginal involvement with functional impairment, refractory to topical treatment
j) Bronchiolitis obliterans not due to other causes
k) Positive liver biopsy; or abnormal liver function tests not due to other causes with alkaline phosphatase >2 x upper limit of normal, AST or ALT >3 x upper limit of normal, or total bilirubin >1.6, and documentation of cGVHD in any organ
l) Positive upper or lower GI biopsy
m) Fasciitis or serositis thought to represent cGVHD and not due to other causes

B. Physical manifestations of Chronic GVHD
Manifestations that are distinctive for chronic GVHD can begin before day 100 after the transplant, and manifestations that are typical of acute GVHD can persist long after day 100. For this reason, the differential diagnosis between acute and chronic GVHD cannot be made solely according to the time interval from transplant. The diagnosis of chronic GVHD requires at least one manifestation that is distinctive for chronic GVHD (*identified by italic print below*) as opposed to acute GVHD. In all cases, infection and others causes must be ruled out in the differential diagnosis of chronic GVHD. Karnofsky or Lansky Clinical Performance scores <60%, ≥15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ system are listed below (*italic print identifies manifestation more distinct of chronic GVHD):*

Skin	Erythema, dryness, pruritis, macular-papular or urticarial rash, *pigmentary changes (i.e., hyperpigmentation, vitiligo), mottling, papulosquamous or lichenoid plaques, hyperkeratosis, exfoliation (ichthyosis), nodules, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)*. The extent of skin involvement and the skin thickness score for patients with scleroderma needs to be recorded at the time of diagnosis, when changes in treatment are made and when assessing treatment response. Medical photos are also useful for assessing the extent of skin involvement and response to treatment.
Nails	*B. Ridging, onychodystrophy, onycholysis*
Hair	*Premature graying (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair*

Mouth	Dryness, burning, gingivitis, mucositis, striae, *dryness, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tightness around the mouth, sensitivity to acidic, strong flavors, heat or cold, tooth decay*
Eyes	*Dryness, burning, blurring, gritty eyes, photophobia, pain*
Vagina/vulva	*Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not induced by ovarian failure or other causes*
Liver	Jaundice and elevated liver function tests not due to other causes (see laboratory tests)
Lung	*Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis*
GI	Anorexia, nausea, vomiting, diarrhea, *malabsorption, dysphagia, odynophagia*
Myofascial	*Stiffness and tightness with restriction of movement, occasionally with swelling, pain, cramping, erythema and induration, most commonly affecting the forearms, wrists and hands, ankles, legs and feet, inability to extend the wrists without flexing the fingers or the elbows, contractures*
Muscle	*Proximal muscle weakness, cramping*
Skeletal	*Arthralgia of large proximal girdle joints and sometimes smaller joints*
Serosal	*Unexplained effusions involving the pleural, pericardial, or peritoneal cavities not due to venocclusive disease of the liver, cardiac insufficiency, malignancy, infection, GM-CSF toxicity or other causes*

C. Laboratory Testing and Diagnostic Indicators of Chronic GVHD

Eye	*Schirmer's test with a mean value ≤ 5 mm at 5 minutes, or values of 6-10 mm in patients who have sicca symptoms, or keratitis detected by slit lamp examination*
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase ≥2 x upper limit, of normal, AST or ALT >3 x upper limit of normal or total serum bilirubin ≥1.6)
Lung	*New obstructive lung defect defined as an FEV_1 <80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV_1/FVC by >12% within a period of less than 1 year, thought not to be caused by an infectious process, asthma or recurrent aspiration from the sinuses or from gastroesophageal reflux. In the absence of GVHD in any other organ, the diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage, evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scan of the lungs, or confirmation by thoracoscopic biopsy.*
Esophagus	*Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry*
Intestine	Endoscopic findings of mucosal edema and erythema or focal erosions with histological changes of apoptotic epithelial cells and crypt cell drop

	out. Patients with unresolved acute GVHD may have more severe intestinal mucosal lesions including ulcers and mucosal sloughing.
Muscle	*Elevated CPK or aldolase, EMG findings consistent with myositis with biopsy revealing no other etiological process*
Blood	Thrombocytopenia (usually 20,000-100,000/□l), eosinophilia (> 0.4 x 10^3/uL), hypogammaglobulinemia. Hypergammaglobulinemia and autoantibodies occur in some cases.

D. Guidelines for Treatment of Chronic GVHD after allogeneic HSCT

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

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

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

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

Appendix H

EVALUATION OF DISEASE RESPONSE

Based on Cheson et al. "Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting standards for Therapeutic Trials in Acute Myeloid Leukemia"
in JCO, Vol 21, No 24 (December 15), 2003: pp 4642-4649

Morphologic Leukemia-free state:
- < 5% blasts in a marrow aspirate with marrow spicules and with a count of at least 200 nucleated cells
- No blasts with Auer rods
- No extramedullary disease

Morphologic Complete remission (CR):
Morphologic Leukemia-free state, AND
- Absolute neutrophil count (ANC) > 1,000/μL
- Platelets ≥ 100,000/μL

Morphologic Complete remission with incomplete count recovery (CRi):
The presence of morphologic CR, except
- ANC < 1,000/μL or
- Platelets < 100,000/μL

Appendix I

STUDY COORDINATOR'S MANUAL

I. Introduction

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

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

II. Institutional Review Board Review of Protocols and Modifications

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

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

III. Registrations

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

IV. Reporting Adverse Events

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

Expedited Reporting Requirements

All adverse events (whether occurring on-site or off-site), which in the opinion of the principal investigator are (1) unexpected, and (2) related or possibly related to the

research and (3) serious or suggests that the research places research participants or others at a greater risk of physical or psychological harm than was previously known or recognized must be submitted to the IRB within ten (10) calendar days of becoming aware of the event.

Definitions
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.

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

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

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

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

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

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

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

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

Procedure for Reporting Serious and Unexpected Adverse Events from Participating Sites

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

Obligation of Investigators

All grade 3 or 4 adverse events (or highly unusual grade 2 adverse events), which occur between start of any protocol intervention and 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 selected (for this protocol) NCI Common Toxicity Criteria(NCI-CTC) version 4 (**Appendix P**). Attributes will include a description, date of onset, maximum severity, and assessment of relationship to the study agent or other suspect agent(s). These grade 3 or 4 adverse events will be reported to the DSMB as part of the biannual review of the protocol. The DSMB report is submitted with the annual IRB renewal.

Reporting of Unanticipated Problems that Involve Risk to Research Participants or Others:

Any incident, experience, or outcome that meets both of the following criteria:

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

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

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

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

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

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

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

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

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

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

APPENDIX J

Fred Hutchinson Cancer Research Center

SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ ☐ FHCRC/SCCA ☐ Other

Gender: ☐ Male ☐ Female Age: _____

FHCRC Principal Investigator: _____

Phone Number: _____ Mailstop: _____

Date of Report: _____

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

Date Study Staff became aware of event: _____

Date Serious Adverse Event Started: _____

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

Adverse Event: _____

_____

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

(Or attach a MedWatch Form or other SAE reporting form if one has been completed.) Use Page 2, if necessary: _____

_____

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

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

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

#1 _____

Pharmaceutical product/medical treatment/procedure

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

#2 _____

Pharmaceutical product/medical treatment/procedure

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

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

Report Completed by: Date:

_____ _____

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

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

_____ _____

Signature of Principal Investigator Date:

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

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ Date of Report: _____

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

Appendix K

NOTICE OF DEATH

Patient ID: _____ Date of Death: _____

Place of Event: _____

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

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Form completed by: _____ Date: _____

Appendix L

PROTOCOL 2430
PATIENT DEMOGRAPHICS AND ELIGIBILITY FORM

Protocol 2430 Patient Demographics and Eligibility Form
Please Fax this completed form to (206)-667-5378 for patient registration.
Questions regarding eligibility should go to Brenda Sandmaier, MD, 206-667-4961

UPN: _____

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

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

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

Patient Diagnosis:
_____

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

Status at Transplant:
_____

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

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

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

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

Protocol 2430 Eligibility

Inclusion Criteria:

Relapse Risk Score

Variable	Score	Patient Score	PI Initial and Date
Unfavorable Cytogenetics	+1		
CR2	+1		
> CR3	+2		
Lack of pre-HCT CBC Recovery	+0.5		
> 18 months between diagnosis and HCT	-2		
Total Risk Score			

Relapse Risk Score ≤ 0: LOW RISK (low risk group terminated August 2014– see statistical section)

Relapse Risk Score > 0: HIGH RISK

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

TBI DOSE LEVEL

☐ 1st dose level: 200 cGy
☐ 2nd dose level: 300 cGy
☐ 3rd dose level: 400 cGy

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

Transplant Center:

_____

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

All of the following questions (2-5) must be marked “Yes” for the patient to enter the study. (Pediatric Attending will determine eligibility of pediatric patients based on criteria outlined in sections “Inclusions” and “Exclusions”)

2a. ☐ Yes ☐ No Patient age ≥ 55 with AML

--OR--

2b. ☐ Yes ☐ No Patient age is >1 and < 55 with AML, who also through pre-existing medical conditions or prior therapy are considered to be at high risk for serious toxicities associated with a conventional, high-dose preparative regimen.

3. ☐ Yes ☐ No Patient is in morphologic leukemia-free state (marrow blasts < 5%) without evidence of extramedullary disease within 21 days of HCT.

4. ☐ Yes ☐ No HLA-identical related or HLA-matched unrelated donor is available

5. ☐ Yes ☐ No ☐ N/A Unrelated donors who are prospectively:

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

AND

b. Only a single allele disparity will be allowed for HLA-A, B, or C as defined by high resolution typing (See appendix O for other donor selection details) **Exclusion Criteria**

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

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

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

d. Yes ☐ No ☐ Patient and donor pairs must not be homozygous at mismatched allele

Exclusion Criteria

Each of the following questions must be marked "No" Or "NA" for the patient to enroll on 2430.

1. ☐ Yes ☐ No AML FAB M3 in CR1

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

3. ☐ Yes ☐ No Active AML involvement of the central nervous system (CNS) with disease refractory to intrathecal chemotherapy. (For LP requirement and intrathecal treatment guidelines, see Appendix N).

4. ☐ Yes ☐ No Presence of circulating leukemic blasts (in the peripheral blood) detected by standard morphology.

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

6. ☐ Yes ☐ No Patient is HIV positive

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

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

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

   PI Signature: _____ **Date**: _____

   Yes ☐ No ☐ **Pulmonary:** DLCO < 40% (corrected), TLC <40%, FEV1 <40% and/or receiving supplementary continuous oxygen.

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

   PI Signature: _____ **Date**: _____

   Yes ☐ No ☐ **Liver Function Abnormalities:** Patients with clinical or laboratory evidence of liver disease would be evaluated for the cause of liver disease, its clinical severity in terms of liver function, and the degree of portal hypertension. Patients will be excluded if they are found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding

esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bridging fibrosis, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3 mg/dL, or symptomatic biliary disease.

Yes ☐ No ☐ **Renal**: Serum Creatinine is outside of the normal limits as specified by institutional guidelines. For patients with serum creatinine > upper limit of normal, a 24-hour creatinine clearance will be performed and should be equal to or more than the lower limit of normal

Creatinine Clearance: _____ **Date**: _____

PI Signature: _____ **Date**: _____

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

9. ☐ 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 will not be allowed within three weeks of the initiation of conditioning.

10. ☐ Yes ☐ No Patient with poorly controlled hypertension and on multiple antihypertensives.

11. ☐ Yes ☐No Karnofsky score < 60 (see **Appendix B**) or Lansky Score <50 (see **Appendix C**)

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

13. ☐ Yes ☐ No Patient has active bacterial or fungal infection unresponsive to medical treatment.

Note – the HCT-Comorbidity score (see Appendix Q) is: _____

Signature of person completing form: _____ Date: _____

Signature of Principal Investigator: _____ Date: _____

Appendix M
CORE CASE REPORT FORMS

CRFsVersion3.pdf

Appendix N
INTRATHECAL THERAPY ADMINISTRATION

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

intrathecaltherapy-combined.pdf

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

hla_testing_donors-recipients.pdf

APPENDIX P
Adapted from
COMMON TOXICITY CRITERIA (CTC)
Version 4.0

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

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

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

Allergic reaction	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated	Death
Immune system disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
INFECTIONS AND INFESTATIONS			
Enterocolitis infectious	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic, endoscopic, or operative intervention indicated; profuse watery diarrhea with signs of hypovolemia; bloody diarrhea; fever; severe abdominal pain; hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Infections and infestations - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
INVESTIGATIONS			
Alanine aminotransferase increased	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Aspartate aminotransferase increased	>5.0 - 20.0 x ULN	>20.0 x ULN	-

Blood bilirubin increased	>3.0 - 10.0 x ULN	>10.0 x ULN	-
Carbon monoxide diffusing capacity decreased	Asymptomatic decrease of >8 units drop; >5 units drop along with the presence of pulmonary symptoms (e.g. , >Grade 2 hypoxia or >Grade 2 or higher dyspnea)	-	-
Cardiac troponin I increased	Levels consistent with myocardial infarction as defined by the manufacturer	-	-
Cardiac troponin T increased	Levels consistent with myocardial infarction as defined by the manufacturer	-	-
Creatinine increased	>3.0 baseline; >3.0 - 6.0 x ULN	>6.0 x ULN	-
Weight gain	>=20% from baseline	-	-

	Grade		
Adverse Event	**3**	**4**	**5**
METABOLISM AND NUTRITIONAL DISORDERS			
Hypercalcemia	Corrected serum calcium of >12.5 - 13.5 mg/dL;>3.1 - 3.4 mmol/L; Ionized calcium >1.6 - 1.8 mmol/L; hospitalization indicated	Corrected serum calcium of >13.5 mg/dL; >3.4 mmol/L; Ionized calcium >1.8 mmol/L; life-threatening consequences	Death
Hypertriglyceridemia	>500 mg/dL - 1000 mg/dL; >5.7 mmol/L - 11.4 mmol/L	>1000 mg/dL; >11.4 mmol/L; life-threatening consequences	Death
Hyperuricemia	>ULN - 10 mg/dL (0.59 mmol/L) with physiologic consequences	>10 mg/dL; >0.59 mmol/L; life-threatening consequences	Death
Tumor lysis syndrome	Present	Life-threatening consequences; urgent intervention indicated	Death

	Grade		
Adverse Event	**3**	**4**	**5**
NEOPLASMS BENIGN, MALIGNANT, AND UNSPECIFIED (INC CYSTS AND POLYPS)			
Treatment related secondary malignancy	Non life-threatening secondary malignancy	Acute life-threatening secondary malignancy; blast crisis in leukemia	Death

Adverse Event	Grade 3	4	5
NERVOUS SYSTEM DISORDERS			
Dysarthria	Severe impairment of articulation or slurred speech	-	-
Intracranial hemorrhage	Ventriculostomy, ICP monitoring, intraventricular thrombolysis, or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Ischemia cerebrovascular	-	-	-
Leukoencephalopathy	Severe symptoms; extensive T2/FLAIR hyperintensities, involving periventricular white matter involving 2/3 or more of susceptible areas of cerebrum +/- moderate to severe increase in SAS and/or moderate to severe ventriculomegaly	Life-threatening consequences; extensive T2/FLAIR hyperintensities, involving periventricular white matter involving most of susceptible areas of cerebrum +/- moderate to severe increase in SAS and/or moderate to severe ventriculomegaly	Death
Seizure	Multiple seizures despite medical intervention	Life-threatening; prolonged repetitive seizures	Death
Syncope	Fainting; orthostatic collapse	-	-
Nervous system disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

Adverse Event	Grade 3	4	5
RENAL AND URINARY DISORDERS			
Chronic kidney disease	eGFR or CrCl 29 - 15 ml/min/1.73 m2	eGFR or CrCl <15 ml/min/1.73 m2; dialysis or renal transplant indicated	Death
Renal and urinary disorders - Other, specify	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated;	Life-threatening consequences; urgent intervention indicated	Death

	disabling; limiting self care ADL		
Grade Adverse Event	**3**	**4**	**5**
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
Grade Adverse Event	**3**	**4**	**5**
RESPIRATORY, THORACIC, AND MEDIASTINAL DISORDERS			
Adult respiratory distress syndrome	Present with radiologic findings; intubation not indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Apnea	Present; medical intervention indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Bronchopulmonary hemorrhage	Transfusion, radiologic, endoscopic, or operative intervention indicated (e.g., hemostasis of bleeding site)	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Hypoxia	Decreased oxygen saturation at rest (e.g., pulse oximeter <88% or PaO2 <=55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Pleural effusion	Symptomatic with respiratory distress and hypoxia; surgical intervention including chest tube or pleurodesis indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Pneumonitis	Severe symptoms; limiting self care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Pulmonary edema	Severe dyspnea or dyspnea at rest; oxygen indicated; limiting self care ADL	Life-threatening respiratory compromise; urgent intervention or intubation with ventilatory support indicated	Death
Respiratory failure	-	Life-threatening consequences; urgent intervention, intubation, or ventilatory support	Death

		indicated	
		Grade	
Adverse Event	**3**	**4**	**5**
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Erythema multiforme	Target lesions covering >30% BSA and associated with oral or genital erosions	Target lesions covering >30% BSA; associated with fluid or electrolyte abnormalities; ICU care or burn unit indicated	Death
		Grade	
Adverse Event	**3**	**4**	**5**
VASCULAR DISORDERS			
Capillary leak syndrome	Severe symptoms; intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Hypotension	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated	Death
Thromboembolic event	Thrombosis (e.g., uncomplicated pulmonary embolism [venous], non-embolic cardiac mural [arterial] thrombus), medical intervention indicated	Life-threatening (e.g., pulmonary embolism, cerebrovascular event, arterial insufficiency); hemodynamic or neurologic instability; urgent intervention indicated	Death
Vasculitis	Severe symptoms, medical intervention indicated (e.g., steroids)	Life-threatening; evidence of peripheral or visceral ischemia; urgent intervention indicated	Death

Appendix Q

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

Assign scores appropriately if the patient has any of these comorbidities

UPN_____ Date_____

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

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

EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Appendix R
Weight / Adjusted Weight for Drug Dosing

weight_for_drug_dosing.pdf

Appendix S
COORDINATING CENTER FUNCTIONS
Outside Center – PI Communication in Hematologic Malignancies

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

a. Study Management:
   1. Each local PI is responsible for selection, training and oversight of local study coordinators
   2. The Coordinating Center registers subjects on the study and assigns study IDs
   3. 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
   4. The quality of data is monitored in an ongoing fashion with the study team and corrective action plans instituted as necessary

b. Data Analysis:
   1. Study staff review data for completeness as it is submitted by the sites
   2. 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:
   1. The trial coordinators at collaborating centers or the local PIs will report SAEs (as defined by the protocol) to the Coordinating Center and an official report of an SAE is faxed to the Coordinating Center within ten days
   2. 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
   3. 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
   4. 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

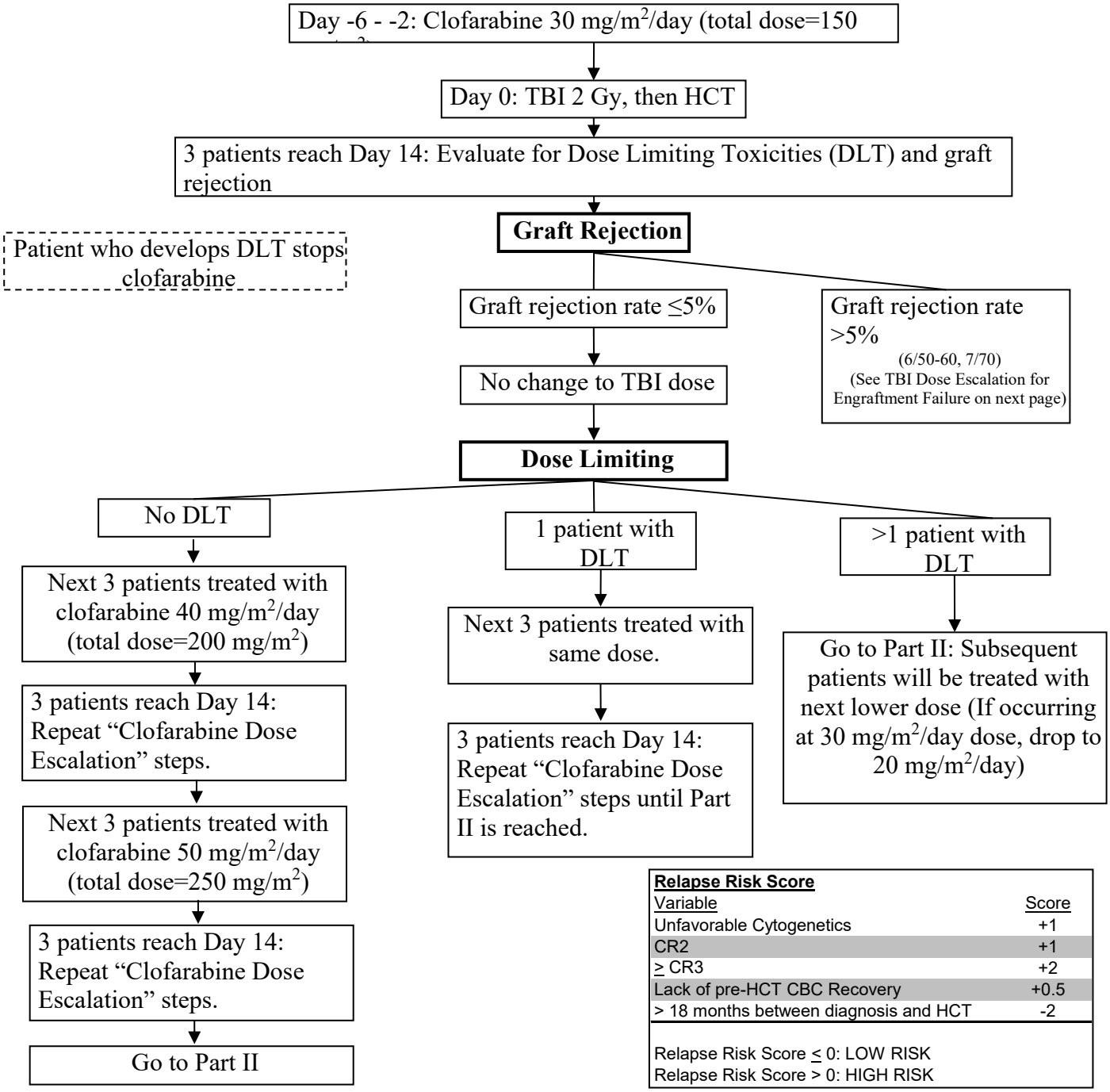
a. The Coordinating Center reviews any modifications to consent forms to ensure that site consents do not delete or change the basic or additional elements or alternative required in the sample consent form

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

a. Subjects must provide written informed consent prior to study participation

b. The Coordinating Center verifies eligibility and signed consent prior to assigning a study ID number

Appendix T
Plan of Treatment in a Flow Chart Format

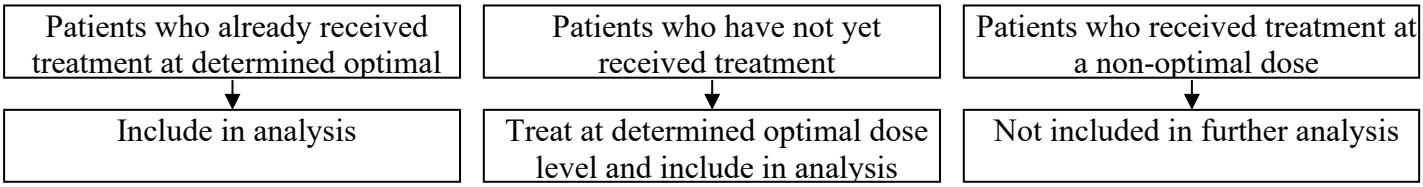


Relapse Risk Score

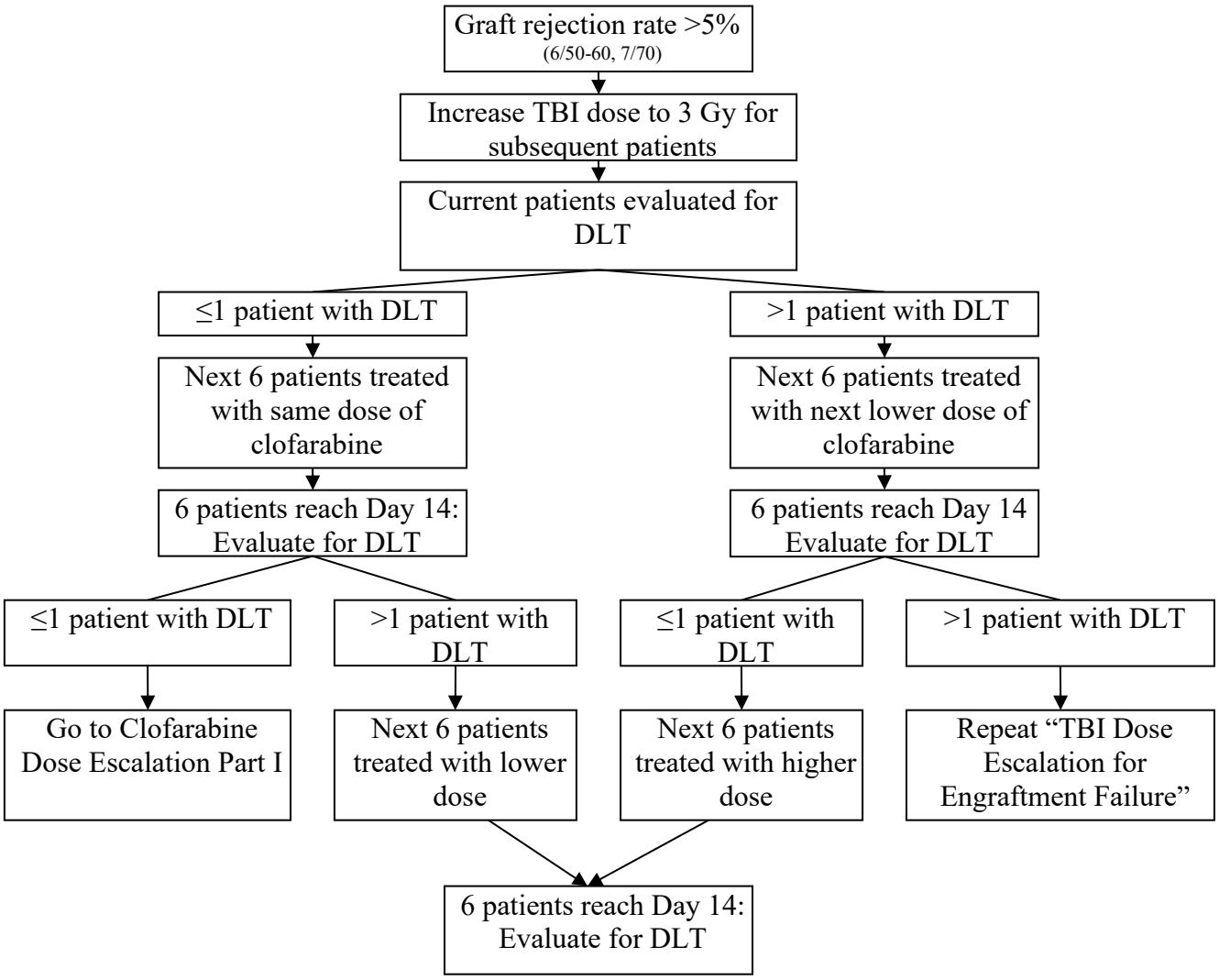
Variable	Score
Unfavorable Cytogenetics	+1
CR2	+1
≥ CR3	+2
Lack of pre-HCT CBC Recovery	+0.5
> 18 months between diagnosis and HCT	-2

Relapse Risk Score ≤ 0: LOW RISK
Relapse Risk Score > 0: HIGH RISK

PART II: Accrual extended to patients with relapse risk ≤0 (low-risk) (low risk group terminated August 2014 – see statistical section) and >0 (high-risk). Analyze as separate groups.



TBI Dose Escalation for Engraftment Failure



Appendix U
Radiotherapy Treatment Guidelines per Standard Practice


TBI_Adult_Non_Myeloablative.pdf


TBI_Pediatric_NON_Myeloablative.pdf