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Title of Protocol:
Multi-center, open-label randomized study of single or double myeloablative cord blood transplantation with or without infusion of off–the-shelf ex vivo expanded cryopreserved cord blood progenitor cells in patients with hematologic malignancies

Investigators List:		
Filippo Milano, MD, PhD	Assistant Member, FHCRC Assistant Professor, UW Principal Investigator (FHCRC)	206-667-5925
Colleen Delaney, MD, MSc	Affiliate Member, FHCRC Associate Professor, Dep't of Pediatric Hematology/Oncology , UW	206-667-1385
Ann Dahlberg, MD	Assistant Member, FHCRC Assistant Professor, Dept't of Pediatric Hematology/Oncology, UW	206-667-1959
Shelly Heimfeld, PhD	Affiliate Member, FHCRC	206-667-4004
Rachel Salit, MD	Assistant Member, FHCRC	206-667-1317
Corinne Summers, MD	Assistant Member, FHCRC Assistant Professor, UW	206-667-3119
Biostatistician:		
Wendy Leisenring, ScD	Member, FHCRC	206-667-4374
Research Staff:		
Nancy Anderson	Research Nurse	206-667-6264
Connie Nakano	Lead Regulatory Affairs Associate	206-667-3011
Denise Ziegler	Study Coordinator	206-667-5762
Mary Joy Lopez	Data Coordinator	206-667-6139

BB-IND 14184 (IND Sponsor: Nohla Therapeutics, Inc.)		
Coordinating Center:		
Fred Hutchinson Cancer Research Center		
Additional Performance Sites:		
Site	Investigator	Phone Number
City of Hope Cancer Center	Chatchada Karanes, MD Clinical Professor of Hematology/HCT, COH	626-359-8111 ext. 62691
Cleveland Clinic	Rabi Hanna, MD Director, Pediatrics Bone Marrow Transplantation Program	216-444-0663
Duke University	Joanne Kurtzberg, MD Professor of Pediatrics and Pathology, Duke	919-668-1100
University of Colorado	Jonathan Gutman, MD Assistant Professor of Medicine, UCD	720-848-0644

	Affiliate Investigator, FHCRC	
Dana-Farber Cancer Institute/Boston Children's Hospital	Christine Duncan, MD, MSc Attending Physician of Medicine, Hematology-Oncology, Boston Children's Hospital Attending Physician of Pediatric Oncology, DFCI	617-632-6255
Stanford University	Andrew Rezvani, MD Assistant Professor of Medicine (Blood and Marrow Transplantation) at the Stanford University Medical Center	650-725-4077
Icahn School of Medicine at Mount Sinai	Alla Keyzner, MD Assistant Professor of Medicine Department of Medicine, Hematology/Medical Oncology, Adult Hematopoietic Stem Cell Transplant Program at Icahn School of Medicine at Mount Sinai	646-942-8201

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1.0 INTRODUCTION

Delayed myeloid engraftment is a known risk factor for CBT recipients and is associated with the low TNC and $CD34^+$ cell doses provided in a single or double CB graft. In fact, a recent analysis of adult single CBT (sCBT) recipients demonstrated that infused $CD34^+$ cell dose is the most important predictor of myeloid engraftment [6]. Furthermore, Brunstein/Delaney et al have shown that non-relapse mortality (NRM) is highest in double CBT (dCBT) recipients when compared to matched and mismatched unrelated donor recipients (figure 1 to the left) [7].

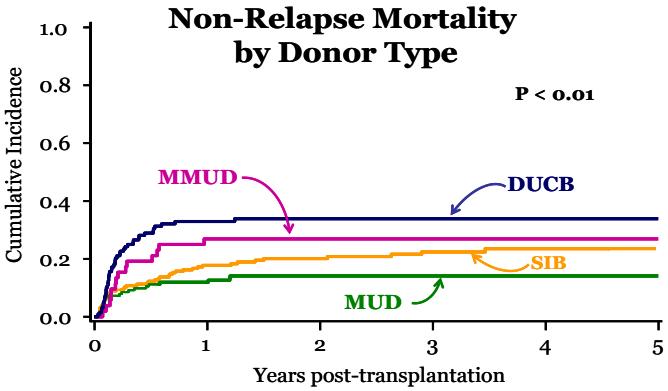


Figure 1. Risk of non-relapse mortality is highest among double CBT recipients. Non-relapse mortality after double CBT (DUCB), matched unrelated donor (MURD), mismatched unrelated donor (MMURD) and matched related donor (SIB) transplant.

The majority of the NRM occurred within the first 100 days post transplant with infection being the most common cause of death. Importantly, an analysis of the risk factors for NRM among dCBT recipients revealed a higher risk in patients with delayed myeloid recovery (time to ANC >500/µl) if the recovery was ≥ 26 days, the median time to engraftment in dCBT recipients. However, when the analysis of risk factors for NRM was restricted to include only those dCBT recipients engrafting before day 26, no difference could be found between the donor sources, emphasizing the important contribution of delayed engraftment to increased risk of NRM. Moreover, an ANC of >100 on any given day post stem cell transplant has been previously shown to be a critical threshold for a decreased risk of mortality before day 100 post transplant [8, 9]. Thus, the significant delay in myeloid recovery that is observed in CBT recipients remains a critical barrier to successful outcomes in the CBT setting. This project aims to overcome this critical problem by further development of an ex vivo expanded cell therapy already shown to be capable of providing rapid early myeloid recovery post CBT.

Using an engineered form of the Notch ligand Delta1 for the ex vivo generation of increased numbers of CB $CD34^+$ stem and progenitor cells, we have previously demonstrated that infusion of our expanded, partially HLA-matched cell product results in a significant reduction in the median time to achieve an absolute neutrophil count of 500/µl to just 14.5 days as compared to a median time of 25 days (p=0.002) in a concurrent cohort of 29 patients undergoing identical treatment but with two non-manipulated CB units. Thus, with established proof of principal, our goal was further development of our cellular therapy as an economically and clinically feasible product with ready patient access for evaluation in pivotal clinical efficacy trials.

Critical to this, and a focus of was the question of whether HLA-matching is required for safe infusion of our product which is devoid of T cells. Without the need for HLA matching, fresh CB units can be collected in collaboration with accredited public CB banks for immediate ex vivo expansion and the final product cryopreserved for future, but immediate, on demand use. In contrast to our initial trial using at least partially HLA-matched CB units for expansion followed by immediate infusion, patient access to the newly proposed expanded CB product would be dramatically enhanced as all of the expanded products banked would be potentially available for any given patient, regardless of HLA typing, race/ethnicity or location of the patient. Moreover, the ability to create an off-the-shelf universal donor expanded cell therapy is not only important to the project proposed herein aimed at shortening the duration of severe neutropenia post CBT, but is likely to enhance more broad areas of investigation outside of stem cell transplantation, e.g., as a way of providing temporary myeloid engraftment for treatment of chemotherapy induced severe neutropenia or accidental radiation exposure.

To first address the question of safety when infusing an off-the-shelf product, we carried out a pilot study, FHCRC Protocol #2378, *Infusion of Off–the-Shelf Ex Vivo Expanded Cryopreserved Cord Blood Progenitor Cells to Augment Single or Double Myeloablative Cord Blood Transplantation in Patients with Hematologic Malignancies.* As discussed more below, no safety issues have been observed, and the ability of the expanded cell product to contribute to early myeloid engraftment has been demonstrated. We are now ready to determine the clinical efficacy of this product in a randomized multi-center trial comparing the time to engraftment in patients

receiving a myeloablative single or double CBT with or without infusion of a non-HLA matched pre-expanded cryopreserved cord blood progenitors.

2.0 BACKGROUND

2.1 Study Disease

Cord blood is an effective and widely used source of stem cells for patients undergoing hematopoietic stem cell transplantation for hematologic malignancies. Most patients now receive two units of CB derived from different donors to better ensure provision of adequate stem cell numbers for reliable donor engraftment. However, with this modest two-fold increase provided by the second CB unit, the time to donor engraftment is still relatively delayed, averaging more than three weeks to achieve adequate numbers of myeloid cells. This leaves patients susceptible to infection and associated morbidity and mortality. Thus, although CB offers some distinct advantages over conventional stem cell sources as it is readily available with no donor attrition and allows reduced stringency in HLA matching without an increase in GVHD thereby increasing the donor pool especially for minority or mixed ethnicity patients, the increased risks of delayed engraftment or graft failure and early transplant related mortality that is associated with the low cell doses in a CB graft remains a barrier to the more wide spread use of this stem cell source. To address this, our laboratory has investigated the role of the Notch signaling pathway in regulating ex vivo expansion of hematopoietic stem/progenitor cells with the goal of generating increased numbers of progenitor cells capable of rapid repopulation in vivo to improve the kinetics of hematopoietic recovery following CBT.

Notch mediated ex vivo expanded cord blood progenitor cells

Using purified $CD34^+$ cord blood progenitor cells cultured in the presence of an engineered form of the Notch ligand Delta1, we have developed a cellular therapy product that may provide rapid temporary myeloid engraftment for prevention of infection and that may potentially facilitate host hematopoietic recovery following high dose therapy. Initial clinical data from our Phase I trial have demonstrated rapid recovery of myeloid cells in hematopoietic cell transplant patients who received partially HLA-mismatched expanded cells along with a second, partially HLA-mismatched but non-manipulated cord blood unit (FHCRC Protocol 2044). We now propose to extend the indication for this cellular based therapy to overcome infectious complications and prolonged neutropenia seen in patients undergoing myeloablative conditioning regimens prior to transplant with one or two partially mismatched non-manipulated units. These “off-the-shelf”, non HLA-matched expanded cord blood progenitors are capable of at least temporarily rescuing hematopoiesis until engraftment can take place.

In preparation for trial using a pre-expanded and cryopreserved product, the methods for selecting and culturing $CD34^+$ cells from fresh cord blood units and cryopreserving the final expanded product for future use as an “off-the-shelf”, non-HLA matched product have been optimized. We have generated pre-clinical data demonstrating the ability to cryopreserve the expanded cell product without loss of in vivo repopulating ability in an immunodeficient mouse model. In addition, work done in a murine model has demonstrated the ability of murine hematopoietic progenitors that have been expanded in the presence of Notch ligand to provide short-term engraftment when transplanted into major H-2 mismatched recipients, and in separate studies, to facilitate recovery of autologous residual stem cells remaining after radiation.

2.2 Preclinical Data

Below, preclinical data supporting the use of ex vivo expanded and cryopreserved cells is presented.

In vivo repopulating ability is retained following cryopreservation of the expanded cell product
A number of validation experiments have been done to optimize cryopreservation methods of the expanded cell product and to determine whether in vivo repopulating ability is retained when the expanded product is cryopreserved for future infusion. Using an immunodeficient mouse model, we evaluated the ability of ex vivo expanded cryopreserved progenitor cells to engraft. As shown below, initial experiments compared in vivo repopulating ability of expanded cells that were directly infused into immunodeficient mice upon harvest versus those that were harvested post expansion and cryopreserved for future use. There were no significant differences

observed in the in vivo repopulating ability of cells that were cultured, cryopreserved (in standard "Allo" media used for hematopoietic cell cryopreservation in the SCCA Cellular Therapy Lab), and then thawed prior to transplant when compared to expanded progenitor cells that were harvested at the end of culture and freshly infused (Figure 1a). Additional experiments have confirmed that repopulating ability of the expanded cell product is retained following cryopreservation. As shown in Figure 1b, all mice that received expanded cryopreserved cells engrafted (defined >0.5% human CD45 in the marrow) with an average overall human engraftment of 8% at 2 weeks post infusion and 7% at 4 weeks post infusion. Lastly, we have compared various thawing methodologies (thaw and wash, thaw and dilute with dextran/HSA and thaw and directly infuse) and have also not seen a significant difference in the three methods evaluated (Figure 1c). Thus, to avoid cell losses, a thaw and dilute protocol will be used.

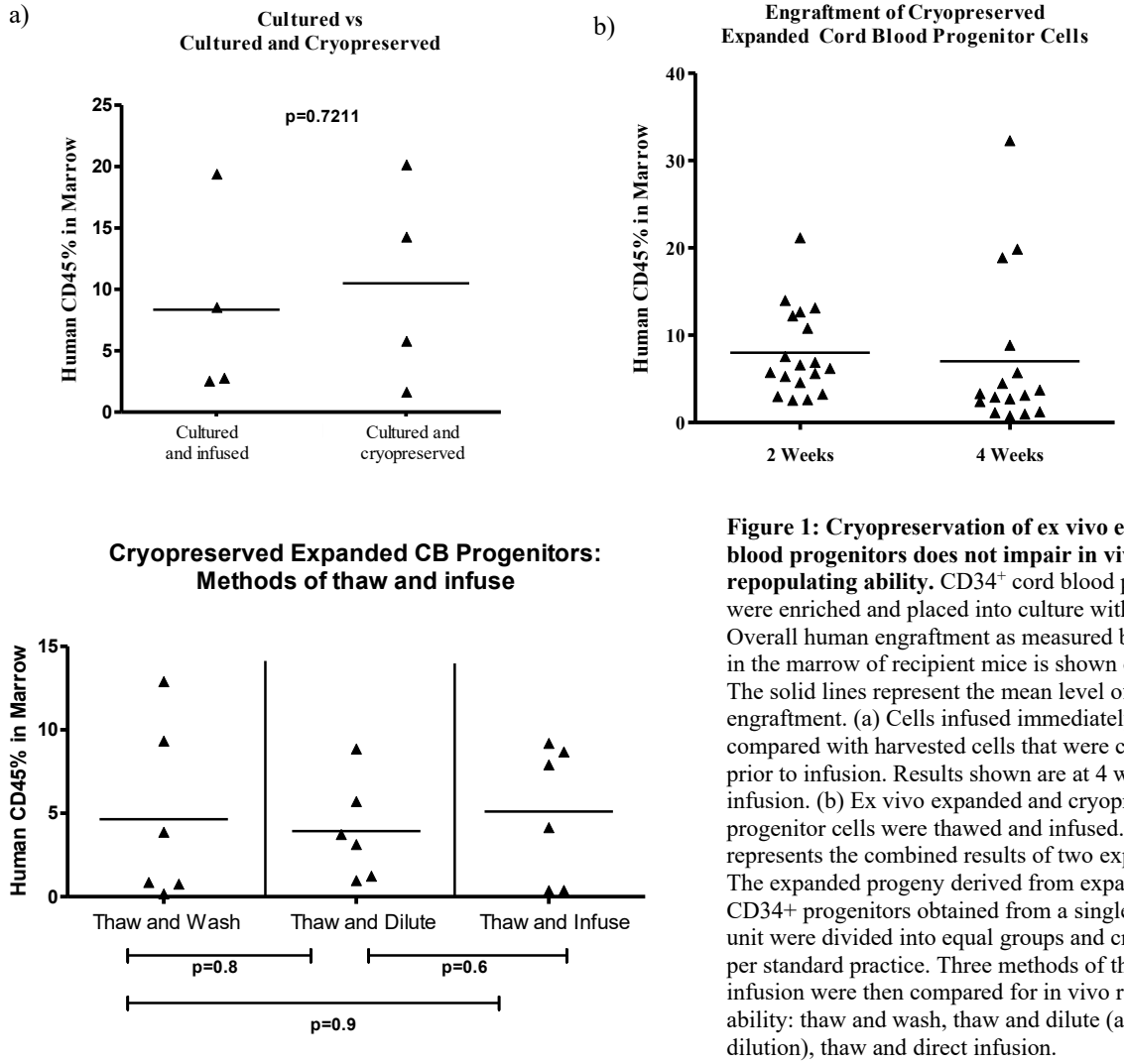


Figure 1: Cryopreservation of ex vivo expanded cord blood progenitors does not impair in vivo repopulating ability. $CD34^+$ cord blood progenitor cells were enriched and placed into culture with $Delta1^{ext\text{-}IgG}$. Overall human engraftment as measured by human CD45 in the marrow of recipient mice is shown on the y axis. The solid lines represent the mean level of human engraftment. (a) Cells infused immediately post culture compared with harvested cells that were cryopreserved prior to infusion. Results shown are at 4 weeks post infusion. (b) Ex vivo expanded and cryopreserved progenitor cells were thawed and infused. The figure represents the combined results of two experiments. (c) The expanded progeny derived from expansion of CD34+ progenitors obtained from a single cord blood unit were divided into equal groups and cryopreserved per standard practice. Three methods of thaw prior to infusion were then compared for in vivo repopulating ability: thaw and wash, thaw and dilute (albumin/dextran dilution), thaw and direct infusion.

Infusion of mismatched ex vivo expanded and cryopreserved hematopoietic progenitors after radiation exposure results in temporary donor hematopoietic recovery and prolongs survival in a murine model

Relevant to the proposed clinical trial, preliminary studies with a murine model have also shown that expanded numbers of progenitor cells derived from murine hematopoietic progenitors (LSK cells) are capable of providing short term engraftment when transplanted in an H-2 mismatched recipient. In this preclinical animal model, LSK cells were isolated by flow cytometry from whole bone marrow (BM) of Ly5a mice (H-2b, CD45.1) and placed in culture in the presence of immobilized Notch ligand. Serum free conditions were used, consisting of Iscove's medium supplemented with cytokines mSCF, hFlt-3 ligand, hIL-6, and hIL-11. After 14 days, expanded cells

were harvested and cryopreserved in 90% FBS + 10% DMSO. On the day of transplant, frozen cells were thawed, washed, and resuspended in PBS + 1% FBS.

To determine the ability of the expanded cells to contribute to in vivo hematopoietic recovery and provide radioprotection in a MHC mismatched model, Balb-c recipient mice (H-2d, CD45.2) were treated with single dose TBI at 7, 7.5 and 8 Gy followed by infusion of either normal saline solution (NS) or 5 x 10^6 expanded cells per mouse. The results presented herein represent the combined data from 3 consecutive experiments, including a total of 150 mice for the generation of survival curves and an additional 54 mice to assess donor engraftment. All animals were observed for 30 days to assess survival. Moribund animals that met specific criteria established by the IACUC were euthanized and counted as an experimental death. Mice contributing to assessment of donor engraftment were sacrificed and peripheral blood and BM collected at 1, 2, and 3 weeks after infusion to determine the in vivo contribution of donor hematopoiesis as determined by flow cytometry.

As shown in figure 2 below, 97% of mice that received NS after a single dose of 8 Gy died in contrast to only 52% mortality in the group treated with 5 x 10^6 expanded cells (despite major MHC differences) ($p<0.0001$). Similarly, at a dose of 7.5 Gy, the survival rate at 30 days was 25% and 70% for mice that received NS and expanded cells, respectively ($p=0.0004$). When the TBI doses was reduced to 7 Gy, all mice infused with expanded cells survived with only one death in the NS group ($p=0.22$) (Figure 2). Of note, no deaths were observed after 30 days. Evaluation of donor derived hematopoiesis demonstrated in vivo persistence of the mismatched expanded cells through day 28, with peak engraftment occurring before day 14. The percent of donor cells in the peripheral blood one week following infusion ranged between 50 to 95% in all mice that received expanded cells, with higher donor engraftment correlating with increased dose of TBI. The level of donor engraftment then decreased over time, but in the surviving mice remained persistent to levels ranging from 10 to 30%. Importantly, no signs of graft versus host disease (GVHD) were observed despite the full-mismatch between the recipient and donor product.

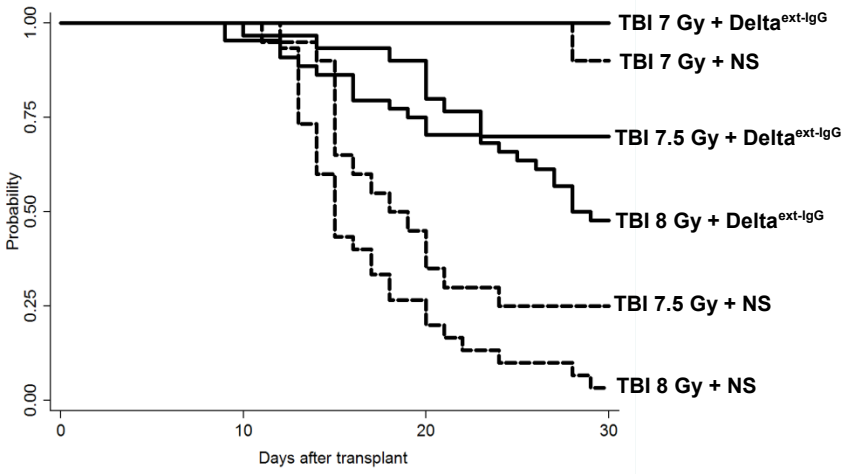


Figure 2. Survival curve of mice irradiated with single dose TBI at 7, 7.5 and 8 Gy and treated with either 5 x 10^6 cryopreserved Delta expanded cells (solid lines) or saline solution (dashed lines).

These studies demonstrated that ex vivo expanded and cryopreserved murine hematopoietic progenitors can be infused safely and effectively to provide temporary donor derived hematopoiesis and prolong survival after acute ionizing radiation. Importantly, these cells can be ex vivo expanded and cryopreserved for future use and retain repopulating ability. Furthermore, in vivo transplantation studies demonstrate that short-term engraftment without manifestations of GVHD can be attained across major H-2 barriers, supporting the use of this product as a universal or third party donor product.

2.3 Initial Clinical Information

Proof of Principal: Preliminary Results of a Phase I CBT Trial Using Ex Vivo Expanded Cord Blood Progenitors
A phase I trial involving transplantation of a non-manipulated partially HLA-matched unit along with CB progenitors from a second partially HLA-matched CB unit that have undergone Notch-mediated ex vivo expansion (FHCRC Protocol 2044) has been conducted. The primary objective was evaluation of safety of

infusing the ex vivo expanded CB progenitors, while secondary objectives included evaluation of the kinetics and durability of hematopoietic reconstitution and the relative contribution to engraftment as provided by the expanded and non-manipulated CB units.
Sixteen patients with high risk acute leukemia in morphologic remission at the time of transplant have been enrolled with a median age of 28.3 years (range, 3.6 to 43.3) and median weight of 66 kilograms (range, 16.5 to 84.7). Based on pioneering studies by Wagner and colleagues at the University of Minnesota demonstrating safety of double CB unit infusion, patients received a myeloablative preparative regimen (1320 cGy TBI, 120mg/kg cytoxan and 75mg/m^2 fludarabine) followed by infusion of one non-manipulated and one ex vivo expanded CB graft. Cord blood donors were chosen on the basis of cell dose and HLA, with all units being ≥ 4/6 matched to the recipient (intermediate resolution at HLA-A and B, and high resolution at DRB1) and at least 3/6 matched to each other. Additional criteria for donor CB selection included a minimum requirement of a total nucleated cell (TNC) dose in the non-manipulated graft of at least 2.5 x 10^7 TNC/kg (based on pre-cryopreservation numbers), independent of the match grade (HLA) of the unit to the recipient. All patients received prophylaxis for graft-versus-host-disease (GVHD) consisting of cyclosporine and mycophenolate mofetil beginning on day -3.

Sixteen days prior to the stem cell infusion date, the unit selected for ex vivo expansion was thawed, clinical grade CD34$^+$ cell selection was performed and cultures initiated in the presence of Notch ligand. On the day of transplant, the cultures were harvested and infused four hours after infusion of a non-cultured, unrelated donor CB graft. At harvest, there was an average fold expansion of CD34$^+$ cells of 184 (range 41-471) and an average fold expansion of total cell numbers of 800 (146 - 1496). Of note, there were no mature T cells infused with the expanded graft. Despite cell losses that occur with CD34 cell selection, in all cases the absolute number of CD34$^+$ cells at the end of culture greatly exceeded the pre-cryopreservation CD34 cell number. The infused CD34$^+$ cell dose derived from the expanded CB graft averaged 12.5 x10^6 CD34/kg (range 0.93 to 49 x10^6, median 8.3 x 10^6).

The kinetics of hematopoietic recovery and the relative contribution of the expanded and non-manipulated CB grafts to engraftment were determined beginning on day +7 post transplant. Time to an initial ANC ≥ 500/µl was evaluable in 14 out of the first 16 patients. In these 14 patients, the time to achieve an absolute neutrophil count of ≥ 500/µl has been shortened significantly, with a median time of 11 days (range, 6 to 34 days). This compares quite favorably with a median time of 25 days (range, 14 to 48 days, p=0.004) in patients undergoing double CB transplantation at our institution with an identical conditioning and post-transplant immune suppression regimen (Figure 3). One patient did experience primary graft rejection due to residual host CD8$^+$ T cell recognition of both the non-manipulated and expanded progenitor CB grafts [2]. Of note, no infusional toxicities or other safety concerns have been observed. Median follow-up time for this set of 16 patients is currently 289 days (range 54-1987), and 8 out of 16 patients remain alive with no evidence of disease, and sustained complete donor engraftment. GVHD has been evaluable in 14 out of 16 patients, and 12 of the 14 were diagnosed with grade II acute GVHD and 2 with grade III. All patients responded to therapy. No chronic extensive GVHD has been observed and three patients have been diagnosed with chronic limited GVHD.

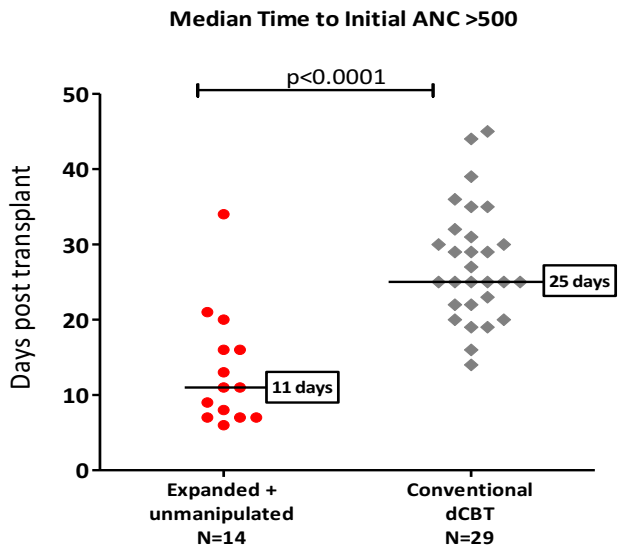


Figure 3. Culture of CB progenitors with Delta1$^{ext-IgG}$ results in more rapid neutrophil recovery in a myeloablative double CBT setting The individual and median times (solid line) to ANC of ≥ 500/µl for patients receiving double unit CBT with two non-manipulated units ("conventional") versus with one ex vivo expanded unit and one non-manipulated unit ("expanded") is presented.

Infusion of third party donor mobilized peripheral blood stem cells to augment CBT
The infusion of ex-vivo expanded cord blood progenitor cells independent of HLA-matching as an "off-the-shelf" product (third party donor product) to overcome delayed hematopoietic recovery in CBT is not dissimilar to an approach taken by MN Fernandez et al., who have demonstrated the safety of infusing T cell depleted third party donor mobilized peripheral blood stem cells as a means to augment the low cell dose in recipients of a single-unit CBT [4]. The third party donor cells were haploidentical in 60% of patients (n=18), but shared no haplotype in 40% (N=12). Using this approach, the incidence of severe GVHD was not increased (and no biopsies obtained for GVHD had detectable DNA from the third party donor) and all patients engrafted with no deaths prior to ANC recovery. The third party donor products infused did contain mature T cells, but were T cell depleted products. Importantly, in the trial proposed herein, $CD34^+$ CB progenitors that have been ex vivo expanded in the presence of Notch ligand do not contain mature T cells as evidenced by immunophenotyping of the harvested product, which should allow for their safe use in a HLA-mismatched setting.

Preliminary Results from a Pilot Study using Off-the-Shelf Expanded Cord Blood Progenitors (FHCRC #2378):
Having previously demonstrated a significant reduction in ANC recovery time in patients receiving these partially HLA-matched expanded cells in a myeloablative CBT, we are now evaluating whether this product could also support rapid myeloid recovery when used as a non-HLA matched, off-the-shelf product. In this pilot study, patients received either a single or double ablative CBT plus infusion of progenitors that have been previously expanded from a fresh CB unit and then cryopreserved for future use. Thirteen patients were enrolled in this pilot study (see Table 1 for patient/graft characteristics). Importantly, no infusional toxicities have been observed and no serious adverse events have been attributed to the off-the-shelf non-HLA matched expanded cord blood progenitor product. All (n=13) patients have engrafted. The median time to achieve an ANC of 100/µl and 500/µl was 12 (range 7 to 23) and 20 days (range 9 to 31), respectively as compared to 19 (range 11 to 37) (p=0.0001) and 25 days (range 14 to 45) (p=0.004) in a concurrent cohort of patients receiving ablative double CBT without the expanded cells. Donor(s)/host chimerism studies were performed weekly from day 7 to 28 on peripheral blood flow sorted into myeloid and lymphoid fractions. Similar to our initial expansion trial using partially HLA-matched expanded CB cells, early (day 7) myelomonocytic (CD33 and CD14) recovery is almost entirely (98–100%) due to cells arising from the expansion product. Cells derived from the expansion product are no longer detected at day 14 in all but 2 patients, which is similar to our previous trial in which contribution to donor engraftment in any cell fraction from the expanded unit was no longer detected by day 14 in half of the patients and only two patients had persistence of the expanded cells beyond day 21. Of note, the expanded cells have not been associated with increased incidence or severity of acute GVHD or with development of alloimmunization. One patient with relapsed disease at the start of conditioning had persistent disease post-transplant and has since died secondary to relapse. Another patient relapsed and is currently being treated. These promising results warrant evaluation in a randomized phase II study to assess clinical efficacy of this non-HLA matched, off-the-shelf expansion product.

Table 1: Unit and Patient Characteristics
Total Enrolled N=13

Patient Characteristics	
Gender, No (%)	
Male	7 (54%)
Female	6 (46%)
Age in years, median (range)	22 (5 to 45)
Weight in kilograms, median (range)	67 (22 to 84)
Diagnosis, No (%)	
ALL	6 (46%)
AML	6 (46%)
MDS	1 (8%
Follow-up in days, median (range)	180 (16 to 385)
Unit Characteristics	
Number of Unmanipulated Donors, No (%)	
1	2 (15%)
2	11 (85%)
HLA match unmanipulated Donors, No (%)	
4/6	8 (62%)
5/6	5 (38%)
Infused Cell Doses (pre-freeze)	
Total Unmanipulated TNC/kg x 10^7	6.2 (4.3 to 17)
Total Unmanipulated CD34/kg x 10^6	0.3 (0.09 to 1)
Expanded Product TNC/kg x 10^7	5.8 (2.2 to 10)
Expanded Product CD34/kg x 10^6	6 (3.1 to 11)

Engraftment Kinetics and Donor Chimerism: As in our previous trial, the kinetics of hematopoietic recovery and the relative contribution of the expanded and unmanipulated grafts to engraftment are determined beginning on day +7 post transplant. All patients treated to date have engrafted. It has been previously demonstrated that an ANC ≥100 is a critical threshold to reduce the risk of death prior to day 100 post hematopoietic cell transplant [8]. Furthermore, in our own single center analysis of severe neutropenia following CBT, modeling both ANC <100 and time to engraftment as a time-dependent covariate correlates significantly with both day 200-TRM and overall survival (unpublished data). In this analysis of 88 patients undergoing CBT, at any given time point, an ANC <100 is associated with a 4.77-fold increase in the risk of overall mortality compared to an ANC >100 (1.74 – 13.11, p=0.002) and an 8.95-fold increase in risk of day 200 transplant related mortality (TRM) (2.59-30.89, p=0.00095). This is similar to findings when modeling the time to engraftment (ANC >500), such that engraftment at a specific time point is associated with a 0.23-fold risk of death as compared to lack of engraftment at this time (0.08 – 0.62, p=0.004) and a 0.11-fold risk of day 200 TRM (0.03 – 0.38, p=0.0005). We therefore evaluated the time to achieve an ANC ≥100 as well as the time to achieve ANC ≥500 in patients undergoing myeloablative dCBT, comparing a concurrent cohort of patients receiving a myeloablative dCBT, our previous trial using partially matched expanded CB units and the initial four patients on this pilot study.

As seen in figure 4 below, the time to achieve an ANC of 100, perhaps the more critical threshold as a threshold for decreased risk of mortality, continues to be significantly shorter in patients receiving our off-the-shelf expanded cell product as compared to conventional dCBT recipients not receiving expanded cells and is similar to what we observed in our previous CB expansion trial using partially matched freshly expanded cord blood progenitors for infusion. With respect to the time to achieve an ANC ≥ 500, the first four patients treated with off-the-shelf progenitors are all evaluable, with a median time to ANC ≥500 of 20 days. Importantly, the results achieved to date compare quite favorably with that seen in 2044 and are supportive for the evaluation of clinical efficacy of this approach compared to conventional CBT in the proposed herein.

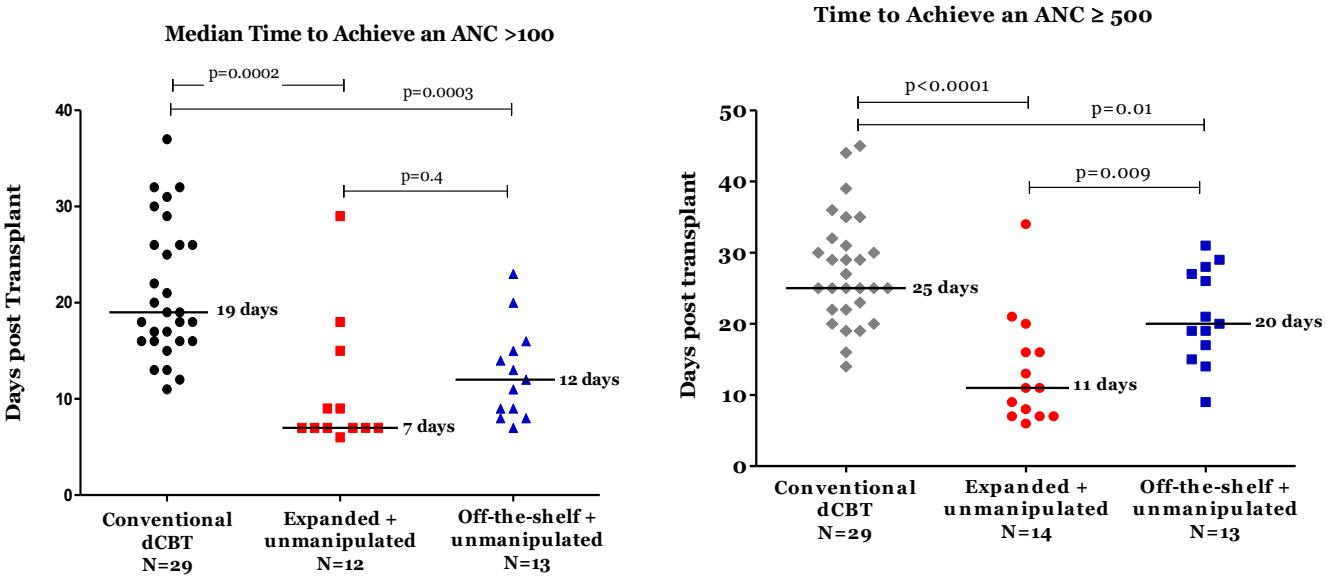


Figure 4: Time to Neutrophil Recovery of 100 and 500 after Myeloablative CBT with and without expanded cells. The time to achieve an ANC of ≥ 100 and 500 in days post transplant is compared in three groups. "Conventional" dCBT recipients (N=29) are those patients receiving a double unmanipulated CB graft with no expanded cell infusion. "Expanded" recipients are patients receiving one unmanipulated graft and one expanded graft, both at least 4/6 HLA-matched to the recipient (N=14). "Off-the-shelf" recipients are patients receiving at least 4/6 HLA-matched unmanipulated CB unit(s) with the addition of a non-HLA matched and off-the-shelf

In order to determine the contribution to donor engraftment in the first month post transplant as derived from the multiple donors and host, recipient peripheral blood is collected weekly from day 7 to 28. The peripheral blood sample is sorted by flow cytometry into lymphoid and myeloid cell fractions (CD33, CD14, CD56, CD3 and CD19) followed by DNA extraction and determination of donor/host chimerism by amplified fragment length polymorphism. An obvious limitation to this approach is the potential lack of sufficient numbers of circulating cells, particularly 7 days after receiving high dose TBI, to enable flow sorting into the various lineages and DNA extraction for donor analysis. However, in the 28 total patients who have received ex vivo expanded cells (either fresh or cryopreserved), day 7 chimerism analysis in all cell fractions, except CD19, was possible in all but 2 patients. Of the two patients that were not evaluable for day 7 chimerism, both received fresh partially matched expanded cell grafts; one patient experienced host mediated graft failure and the other did not have any contribution to donor engraftment from the expanded cells in the setting of early onset HHV6 viremia.

This is in striking contrast to our attempts to collect day 7 donor chimerism data from patients undergoing conventional myeloablative dCBT, where essentially the only chimerism data reliably obtained at this early time point is CD3 chimerism, likely partially reflecting the CD3 cells infused with the graft. However, similar to our initial expansion trial using partially matched expanded CB cells, determination of day 7 peripheral blood chimerism has been possible on all cell fractions except CD19, suggesting that despite low overall counts as reported in the daily clinical CBCs, there are higher absolute numbers of circulating cells in patients receiving ex vivo expanded cell products. Again, early myelomonocytic recovery in patients receiving expanded products is almost entirely (98 – 100%) due to circulating cells as contributed from the off-the-shelf product. However, by day 14, the off-the-shelf product is no longer detected by peripheral blood chimerism analysis in all but two patients who have since converted to 100% donor from an unmanipulated unit (see Figure 5 below). This is not dissimilar to our previous trial in which contribution to donor engraftment in any cell fraction from the expanded unit was no longer detected by day 14 in 5 of 9 evaluable patients. Only two patients had persistence of the expanded cells beyond day 21, but contribution to CD3 engraftment was never observed from the expanded cell graft. One of the two patients died at approximately 6 months post transplant from sepsis/multi-system organ failure, still with engraftment from both her unmanipulated and expanded units and the other patient converted to 100% donor engraftment from the unmanipulated unit at approximately one year post transplant.

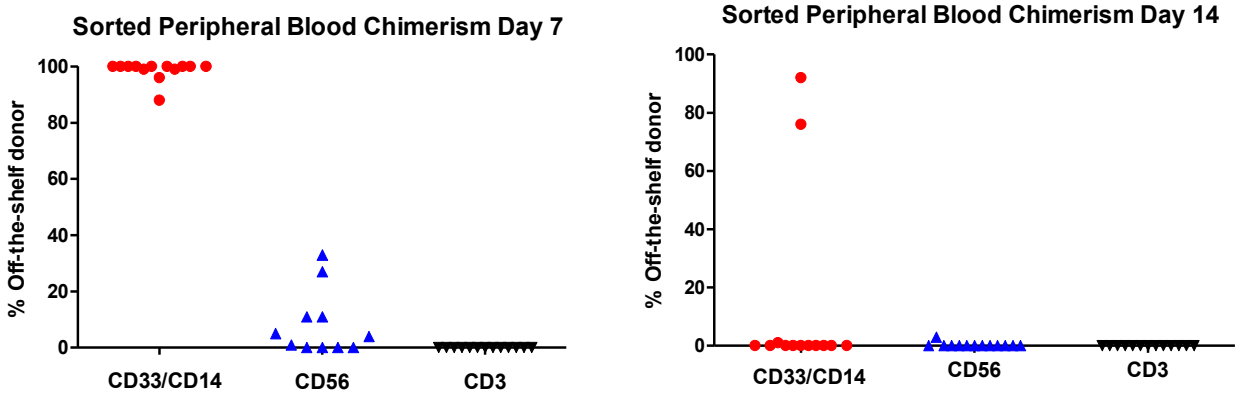


Figure 5. Peripheral blood chimerism day 7 and 14, FHCRC Protocol 2378

2.4 Investigational Agent

Biological Representation

The ex vivo expanded cord blood product ("Delta1-cultured Cryopreserved Umbilical Cord Blood Cells", or DCC-UCB), is derived by culture of enriched $CD34^+$ progenitor cells isolated from a single donor umbilical cord blood (UCB) unit in the presence of recombinant cytokines and the Notch ligand $Delta1^{ext\text{-}IgG}$ for a period of 14 to 16 days. At the end of the culture period, the resulting cell population that makes up the DCC-UCB cells is heterogeneous, consisting of $CD34^+$ progenitor cells and more mature myeloid and lymphoid precursors, as evidenced by flow cytometric analysis for the presence of CD34, CD7, CD14, and CD56 antigens. Typical flow cytometry characterization of DCC-UCB cells is presented in Table 2 below.

Table 2: DCC-UCB Cell Phenotype (N= 80)

	Median Percent	Range
CD34	10.4	4.9 - 24.6
CD7	8.5	2.3 - 25.2
CD14	7.0	0.9 - 22.2
CD15	39.3	0.3 - 65.5
$CD34^+/56^+$	2.6	0.2 - 9.2
$CD3^-/CD16^+/56^+$	4.8	0.7 - 13.0
CD20	0.1	0.0 - 10.2
$CD3^+/CD4^+$	0.3	0.0 - 2.9
$CD3^+/CD8^+$	0.0	0.0 - 0.5
TNC Fold Expansion	1018	213 - 3333
CD34 Fold Expansion	129	23 - 392

There is significant increase of $CD34^+$ and total cell numbers during the culture period, ranging from 23 to 392 fold expansion of $CD34^+$ cells and 213 to 3333 fold expansion of total cell numbers (N= 80 individual cord blood units, processed per defined clinical expansion procedures). Notably, there is a lack of T cells as measured by immunophenotyping. Functionally, this expanded cord blood product is capable of multi-lineage human hematopoietic engraftment in a NOD/SCID mouse model.

2.5 Regimen Rationale

As discussed above, it is important to note that the expanded cell product contains no mature T cells, and therefore should be able to be infused in a recipient who has undergone an intensive preparative regimen. As in the previous trial, we do not anticipate that the expanded unit will be the long term engrafting unit, due to likely eventual (or immediate) rejection mediated by the non-manipulated unit(s). No safety issues infusing these products have been identified in a previously conducted pilot study, and we are therefore now evaluating the clinical efficacy of this product in a randomized multi-center trial comparing the time to engraftment in the patients undergoing myeloablative CBT with or without infusion of this off-the-shelf product.

This clinical trial will allow variation in preparative regimen (see Section 9.2) but will have identical regimen for GVHD prophylaxis. The protocol allowed conditioning regimens are:

1. High dose TBI + Fludarabine + cyclophosphamide
2. Middle intensity TBI + Fludarabine + cyclophosphamide + thiotepa

The additional regimen (middle intensity TBI + Fludarabine + cyclophosphamide + thiotepa) was chosen in addition to the more conventional high dose TBI regimen that was used in our pilot study based on the extensive work published by Dr. Juliet Barker at the Memorial Sloan Kettering Cancer Center[10].This novel regimen was originally developed as an alternative intensive conditioning regimen aimed at reducing the increased risk of lethal regimen-related organ toxicity that has been associated with the more conventional high dose TBI-based regimen used for myeloablative CBT (using 13.2 Gy TBI). In fact, the known risk of end organ toxicity with high dose TBI has led to restriction of this regimen to those 45 years and younger. Although there are nonmyeloablative conditioning regimens that have been used in the CBT setting for those patients older than 45 years, nonmyeloablative conditioning itself is limited by increased risk of graft rejection and relapse in this setting. Thus, there was a significant need to develop a well-tolerated middle intensity, near myeloablative regimen to address the limitations of the high dose TBI and nonmyeloablative regimens. This is particularly true

for those patients older than 45 years or younger patients with significant pre-existing co-morbidities precluding them from the conventional high dose TBI regimen.

Dr. Barker published on this regimen for the first time in 2013 which presented results for 30 patients with acute leukemia and MDS, with the majority having acute leukemia (n=26) [10]. The median age was 56 years (range 18 to 69). The overall incidence of engraftment was 97% (1 graft failure), with a median time to engraftment of 26 days (range, 13 to 43).

Expanding our clinical trial to allow the option of this new conditioning regimen as an alternative to the conventional high dose TBI regimen, with corresponding expanded age range, is strongly motivated by arguments for the importance of generalizability and for the ability to pool results:

- The results of the Barker trial reveal that there is a promising alternative to high dose TBI based conditioning that expands the benefits of a conditioning regimen to a wider cohort, in particular to patients 50 years and older who are otherwise reasonably fit. Furthermore, it is thought to represent a promising alternative to high dose TBI based conditioning.

- The Barker trial results reveal that time to median engraftment using this regimen in an adult population (18 – 69 years) is nearly identical to the setting of CBT using the high dose TBI based regimen currently in use in Protocol 2603. Furthermore, the reported rate of TRM at day 180 was 20%, similar to the conventional high dose TBI regimen. Therefore, it appears that there is a similar unmet need across these settings to reduce median time to engraftment that is a median of 26 days across these settings. Data showing that engraftment times are similar to the conventional high dose TBI regimen are presented in Figure 6 below.

- Importantly, it is reasonable that efficacy of the experimental cell therapy product would be similar across these conditioning regimen. Specifically, the experimental cell therapy product under investigation in this trial is thought to have a mechanism of action that is independent of the conditioning regimen. This is based on preliminary efficacy data demonstrating a reduction of infections and bridging hematopoiesis even in patients receiving this product to mitigate chemotherapy induced neutropenia (i.e., in the non-transplant setting).[11]

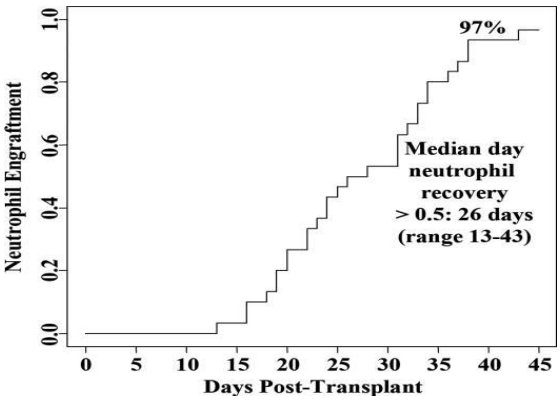


Figure 6. Time to median engraftment using middle-intensity regimen *(Ponce D, et al, BBMT 2013)*

For these reasons, the inclusion of this new middle-intensity regimen and the inclusion of patients up to age 65 enables a clinically well motivated generalization of the assessment of the experimental cell therapy product, and it is scientifically well motivated to use of the same primary endpoint of engraftment across these settings. The use of the same secondary endpoints across these settings also is well motivated.

The regimen selection for individual patients is flexible to allow for attending physician and site preference. There are some absolute criteria, for example, that patients between the ages of 46 and 65 would have to go on the middle-intensity regimen. However, outside of this, the decision of which conditioning regimen would be based on the underlying disease, presence of MRD, age, co-morbidities and attending preference. Furthermore, there is also a move away from high dose TBI by some physicians/centers in younger patients to avoid the long term sequelae of high dose TBI. Because both regimens result in an increased risk of delayed hematopoietic recovery and early transplant related mortality, there will be other patient specific factors that lead to the specific regimen choice by the physician. In general, it is anticipated that younger patients would continue to accrue to the high dose TBI regimen, especially those with minimal residual disease and the middle-intensity regimen would accrue those patients between 46 and 65 years of age and patients for whom the attending physician feels this is the more appropriate regimen.

Supportive care will be in accordance with institutional guidelines to our previous cord blood expansion protocols (FHCRC #2044 and #2378). As in FHCRC Protocols 2044 and 2378, stopping rules for GVHD, infusional toxicity and graft failure will also be in place. The major differences are donor selection for the expanded cell product and the use of one or two unmanipulated units for the unmanipulated cell graft (allowing for a single or double cord blood transplant with infusion of expanded cryopreserved cord blood progenitor cells as adjunctive treatment).

In this study, we will choose the unmanipulated CB donors per a CB selection algorithm (see Section 6.1) allowing a single or double non-manipulated cell graft, and randomize the patients then to receive the additional off-the-shelf product or not.

2.6 Risks/Benefits

Potential Risks
As an off-the-shelf, non-HLA matched cellular therapy, theoretical concerns regarding the infusion of expanded cells exist, including the possibility of infusional toxicity, transfusion associated GVHD, alloimmunization, and immediate rejection of the expanded product. There have been no infusional toxicities associated with the infusion of total nucleated cells (TNC) ranging in dose from $2.8x10^7$/kg to $13.9x10^7$/kg ($CD34^+$ dose ranging from $0.12x10^6$/kg to $13.4x10^6$/kg) in our current Phase I clinical trial. In our pilot study, no infusional toxicities were observed in the first four patients either, with TNC up to 9×10^7/kg from the expanded product. Patients enrolled in this protocol will have been treated with a standard of care myeloablative preparative regimen; however, the expanded cell product will be an off-the-shelf product selected without respect to HLA. Transfusion associated GVHD is unlikely as the expanded product has been $CD34^+$ selected (and therefore T cell depleted) and ex vivo expanded prior to cryopreservation and no mature T cells are generated in culture. Alloimmunization is a definite possibility, but because the expanded cells will come from only one donor at a time, if alloimmunization develops, it will be to only a limited number of HLA antigens and this will be monitored. Lastly, cord blood donor selection for the unmanipulated units for all patients enrolled in this protocol will follow the current umbilical cord blood graft selection algorithm (Section 6.1) that is based on both HLA-typing and cell dose, with 1 or 2 cord blood units selected to achieve the required cell dose. Thus, all patients will have an adequate graft to undergo a cord blood transplant even without the expanded cells. The expanded unit, in this case, is used to augment this conventional graft with the goal of providing rapid but transient myeloid recovery. If there is near immediate immune-mediated rejection of the expanded cell product, it is anticipated that this would not impact patient safety above and beyond the risk inherent in conventional myeloablative cord blood transplantation.

Potential Benefits
Extensive research has been done to define the optimal conditions necessary for *ex vivo* expansion of hematopoietic stem cells (HSC), and various expansion techniques have been developed for this purpose [5]. Despite a lack of convincing data that pluripotent stem cells increase in number and retain an ability to engraft and sustain multi-lineage hematopoiesis in a myeloablated recipient following *ex vivo* expansion, we have focused on the use of an extrinsic regulator of stem cell fate (Notch) to manipulate cell fate decisions ex vivo and have convincingly demonstrated that Notch-mediated expansion of CB stem/progenitor cells results in a dramatic expansion of hematopoietic precursors capable of at least short term myeloid engraftment when infused in a clinical cord blood transplant setting Thus, the preliminary results from our Phase I trial provide the first evidence that a key regulator of cell fate, the Notch pathway, can be used in a clinically compliant manner to generate a safe and potentially efficacious cell therapy product.

Currently, our focus is on overcoming the delay in engraftment experienced by patients undergoing a cord blood transplant, with the goal of improving patient outcomes. The use of CB as a source of hematopoietic stem cells for transplantation offers many advantages, especially for those patients of minority or mixed ethnicity background (and 30% of Caucasians) who cannot identify a suitably matched adult volunteer donor. However, as discussed above, having already demonstrated the ability of this expanded product to engraft at least temporarily in a myeloablative cord blood transplant setting, a critical question becomes whether this product which is devoid of mature T cells can be safely infused in a non-HLA matched setting. While this should be possible due to the lack of mature T cells in the expanded cell population, especially in a myeloablated host, there is also the potential for

rejection of the infused expanded product prior to therapeutic benefit. If we can demonstrate the safety of this approach, the potential impact is tremendous, not just for patients undergoing cord blood transplantation but also for individuals who experience chemotherapy or radiation induced aplasia.

3.0 STUDY OBJECTIVES

3.1 Primary Objectives

Compare the time to neutrophil engraftment (ANC ≥500) in patients receiving a standard of care myeloablative CBT augmented with an off-the-shelf pre-expanded and cryopreserved cord blood product to those who do not receive the product.

3.2 Secondary and Exploratory Objectives

Provide initial data on clinical and economic benefit, such as time to platelet engraftment, duration of initial hospitalization, transplant-related mortality (TRM), death without engraftment, and incidence of severe infections in the first 100 days post transplant. The kinetics of immune system recovery will also be evaluated in both arms.

4.0 STUDY DESIGN

4.1 Description of Study

This Phase II trial is a multi-center, open-label randomized study of single or double myeloablative cord blood transplantation with or without infusion of off–the-shelf ex vivo expanded cryopreserved cord blood progenitor cells in patients with hematologic malignancies

Primary Endpoint

Time to engraftment (ANC ≥500) in both arms (standard myeloablative CBT with and without off-the-shelf expanded cord blood progenitors).

4.2 Secondary Endpoints

1. Platelet engraftment (20k)
2. Incidence of infectious complications in the first 100 days post transplant
3. Overall Survival
4. Non-relapse mortality
5. Acute and chronic GVHD.

4.3 Exploratory Endpoints

1. In vivo persistence of the ex vivo expanded cord blood product
2. Duration of initial hospitalization
3. Grade ≥ 3 infusional toxicity
4. Graft failure: Primary and secondary (see protocol section 13.0 for definition of graft failure)
5. Immune reconstitution: TCR sequencing (see protocol section 10.9)

5.0 PATIENT SELECTION

5.1 Inclusion Criteria

5.1.1 Age Criteria

1. High dose TBI regimen: 6 months to ≤ 45 years
2. Middle intensity TBI regimen: 6 months to ≤ 65 years

Conditioning regimen selection should be based on the underlying disease, presence of MRD, age, co-morbidities, attending physician, and site preference. Conditioning regimen will not require stratification of the randomization due to heterogeneity in the cohort of eligible patients.

5.1.2 Disease Criteria

A. Acute Myeloid Leukemia, including Biphenotypic Acute Leukemia or Mixed-Lineage Leukemia
   1. All patients must have AML that is considered best treated by stem cell transplant by the referring physician and the attending transplant physician.
   2. All patients must be in CR as defined by < 5% blasts by morphology/flow cytometry in a representative bone marrow sample with cellularity ≥ 15% for age.
   3. Patients in which adequate marrow/biopsy specimens cannot be obtained to determine remission status by morphologic assessment, but have fulfilled criteria of remission by flow cytometry, recovery of peripheral blood counts with no circulating blasts, and/or normal cytogenetics (if applicable) may still be eligible. Reasonable attempts must be made to obtain an adequate specimen for morphologic assessment, including possible repeat procedures. These patients must be discussed with the Principal Investigator, Filippo Milano (206-667-5925 or pager, 206-314-1037) prior to enrollment.

B. Acute Lymphoblastic Leukemia, including Biphenotypic Acute Leukemia or Mixed-Lineage Leukemia
   1. High risk CR1 [for example, but not limited to: t(9;22), t(1;19), t(4;11) or other MLL rearrangements, hyplodiploid] or HR as defined by referring institution treatment protocol, greater than 1 cycle to obtain CR; CR2 or greater.
   2. All patients must be in CR as defined by <5% blasts by morphology/flow cytometry in a representative bone marrow sample with cellularity ≥ 15% for age.
   3. Patients in which adequate marrow/biopsy specimens cannot be obtained to determine remission status by morphologic assessment, but have fulfilled criteria of remission by flow cytometry, recovery of peripheral blood counts with no circulating blasts, and/or normal cytogenetics (if applicable) may still be eligible. Reasonable attempts must be made to obtain an adequate specimen for morphologic assessment, including possible repeat procedures. These patients must be discussed with the Principal Investigator, (206-667-5925 or pager, 206-314-1037) prior to enrollment.

C. Chronic myelogenous leukemia excluding refractory blast crisis. To be eligible in first chronic phase (CP1) patient must have failed or be intolerant to tyrosine kinase inhibitor therapy.

D. Myelodysplasia (MDS) IPSS Int-2 or High risk (i.e., RAEB, RAEBt) or refractory anemia with severe pancytopenia or high risk cytogenetics. Blasts must be < 10% by a representative bone marrow aspirate morphology.

5.1.3 Organ Function and Performance Status Criteria

A. Performance status score
   1. Karnofsky (≥ 16 years old) ≥ 70 or ECOG 0-1
   2. Lansky (<16 years old) ≥ 60

B. Renal Function
   1. Adults: Calculated creatinine clearance must be > 60 mL and serum creatinine ≤ 2 mg/dL.
   2. Children (<18 years old): Calculated creatinine clearance must be > 60 mL/min

C. Hepatic Function
   1. Total serum bilirubin must be <3mg/dL unless the elevation is thought to be due to Gilbert's disease or hemolysis.
   2. Transaminases must be < 3x the upper limit of normal per reference values of referring institution

D. Pulmonary function
   1. DLCO corrected >60% normal
   2. For pediatric patients unable to perform pulmonary function tests, O2 saturation >92% on room air

3. May not be on supplemental oxygen.

E. Cardiac function
   1. Left ventricular ejection fraction >45% OR
   2. Shortening fraction > 26%

F. Ability to understand and the willingness to sign a written informed consent document

5.2 Exclusion Criteria

A. Uncontrolled viral or bacterial infection at the time of study enrollment

B. Active or recent (prior 6 month) invasive fungal infection without ID consult and approval

C. History of HIV infection

D. Pregnant or breastfeeding

E. Prior myeloablative transplant containing full dose TBI (greater than 8 Gy).

F. CNS leukemic involvement not clearing with intrathecal chemotherapy and/or cranial radiation prior to initiation of conditioning. Diagnostic lumbar puncture is to be performed as per section 9.7.

G. Patients $\geq$ 45 years: comorbidity score of 5 or higher

6.0 Cord Blood Graft Selection

6.1 Unmanipulated Unit(s)

CB donor selection will be based on institutional guidelines and in general should be selected to optimize both HLA match and cell dose. Additionally, CB grafts shall consist of one or two CB donors based on, but not exclusively determined by, cell dose (TNC/kg and CD34/kg), HLA matching and disease status and indication for transplant. Attending preference will be allowed for single versus double unit as well as the degree of mismatching based on patient specific factors, as long as the following minimum criteria are met:

A. HLA matching:
   i. Minimum requirement: The CB graft(s) must be matched at a minimum at 4/6 HLA-A, B, DRB1 loci with the recipient. Therefore 0-2 mismatches at the A or B or DRB1 loci. based on intermediate resolution A, B antigen and DRB1 allele typing for determination of HLA-match is allowed.
   ii. HLA-matching determined by high resolution typing is allowed per institutional guidelines as long as the minimum (A.i., above) are met.

B. Selection of two CB units is mandatory when a single cord blood unit does not meet the following in the table below.

	Single Unit Allowed for:
Match Grade	**TNC Dose**
6/6	$\geq 2.5 \times 10^7$/kg
5/6, 4/6	$\geq 4.0 (\pm 0.5) \times 10^7$/kg

If two CB units are used the total cell dose of the combined units must be at least 3.0×10^7 TNC per kilogram recipient weight based on pre-cryopreservation numbers, with each CB unit containing a MINIMUM of 1.5×10^7 TNC/kg.

C. The minimum *recommende*d CD34/kg cell dose should be 2×10^5 CD34/kg, total dose from a single or combined double.

D. The unmanipulated CB unit(s) will be FDA licensed or will be obtained under a separate IND, such as the National Marrow Donor Program (NMDP) Protocol 10-CBA conducted under BB IND-7555 or another IND sponsored by (1) a participating institution or (2) an investigator at FHCRC or one of the participating institutions.

E. **FHCRC only:** Up to 5% of the unmanipulated cord blood product (s), when ready for infusion, may be withheld for research purposes as long as thresholds for infused TNC dose are met. These products will be used to conduct studies involving the kinetics of engraftment and immunobiology of double cord transplantation.

6.2 Ex Vivo Expanded Unit (DCC-UCB - Investigational Agent)

The expanded cord blood progenitors to be used for this trial will be selected from a bank of previously expanded cord blood progenitors (per IND 14184) that have been cryopreserved for future clinical research use at FHCRC. Each individual progenitor cell product is derived from a single cord blood unit (donor) that undergoes CD34 selection (and is therefore a T cell depleted product) and is then placed into culture and ex vivo expanded in the presence of Notch ligand (see background). The final product is the total progeny derived from the starting CD34+ cell population which is harvested on day 14 – 16 of culture and then cryopreserved. The fresh cord blood units used for expansion are obtained through collaborations with accredited public cord blood banks. These public cord blood banks provide appropriately screened cord blood units meeting donor eligibility criteria per 21 CFR 1271.

Product selection will be performed for each enrolled patient by the FHCRC PI or qualified designee and product will be shipped to the participating site for outside patients. Refer to shipping details and handling instructions in the FHCRC Protocol 2603: Receipt and Handling of NLA101/DCC-UCB (Delta1-Cultured Cryopreserved Umbilical Cord Blood Cells) Information Sheet (June 2017). Selection of the expanded cord blood progenitor product will be based on the following:

A. Panel Reactive Antibody (PRA) is to be performed on all enrolled patients, and product will be selected based on the specificity of donor directed antibodies when present as possible. PRA negative patients may receive any product that fits cell dose restrictions. HLA matching will not be considered outside of PRA+ patients.

B. Cell Doses:
   1. Infused TNC/kg cell dose will not exceed 1 x 10^8 TNC/kg recipient body weight based on final viable TNC count at the time of cryopreservation.
   2. CD34 cell dose: No upper limit will be placed on the CD34 cells/kg infused.
   3. All expanded products are evaluated by immunophenotyping for the presence of $CD3^+$ cells prior to freezing. While there has been no convincing evidence of a $CD3^+$ cell population, if a product has evidence of a T cell ($CD3^+$) population, this product will not be used unless the dose of $CD3^+$ cells is $<5x10^5$ CD3 cells/kg (recipient weight) based on pre-freeze assessment of CD3 content in the final product.
   4. The expected volume of the ex vivo expanded product is 120 mL after thaw and dilute methodology.

7.0 INFORMED CONSENT OF SUBJECT AND DONOR

The patient and donor units will be completely evaluated. The protocol will be discussed thoroughly with the patient and family, including requirement for data collection and release of medical records and all known risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible and the risks and hazards of the procedure explained to the subject or, in the case of minors, to the subject's responsible family members. Consent will be obtained using forms approved by the Institutional Review Board. A summary of the conference will be dictated for the medical record detailing what was covered.

8.0 SUBJECT REGISTRATION

Informed consent must be signed prior to the performance of any study related procedures or assessments.

FHCRC patients: Eligible subjects will be identified and registered into the system by the Clinical Coordinators Office (CCO) (Intake Office) and assigned a UPN (Unique Patient Number). The CCO will register the subject on to the protocol through the Data Management Office.

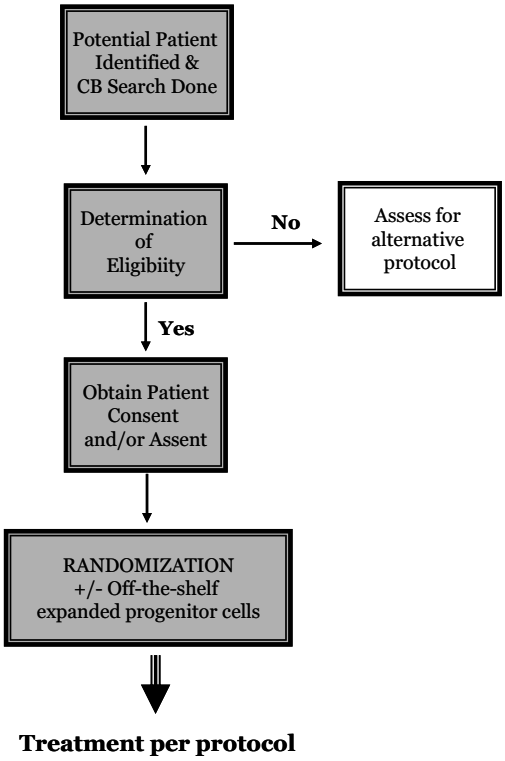
Collaborating institutions: Eligible patients will be identified by the principal investigators of the collaborating institutions who will register the patient with the FHCRC Coordinating Center. Registration will include completion of the eligibility checklist/demographic form provided in the CRF packet. This form will be submitted to the Coordinating Center along with source documents and eligibility will be confirmed by the Coordinating Center PI or qualified designee before enrollment occurs. Questions regarding eligibility or protocol information should be directed to Filippo Milano, MD (206-667-5925).

9.0 TREATMENT PLAN

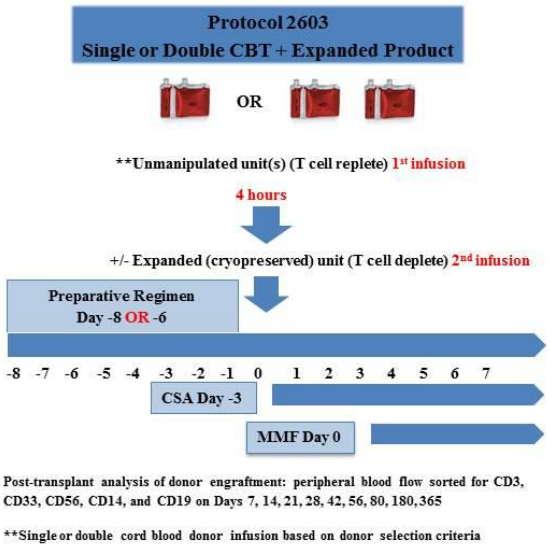
9.1 Treatment Schema

This schema is a pictorial overview only; please see narrative Treatment and Evaluation sections of the protocol for specifics and allowable deviations from time frames shown.

Pre-transplant Determination of Eligibility and Randomization



Treatment Schema



9.2 Conditioning Regimens

There are two conditioning regimens allowed per protocol. They are:

1. Regimen A: High dose TBI + Fludarabine + cyclophosphamide
2. Regimen B: Middle intensity TBI + Fludarabine + cyclophosphamide + thiotepa

REGIMEN A: High Dose TBI regimen (included ages: 6 months through 45 years old)

Fludarabine total 75 mg/m^2 (25 mg/m^2/day IV x 3 days, days -8 to -6), Cyclophosphamide total 120 mg/kg (60 mg/kg IV x 2 days, days -7 to -6), High Dose TBI total 1320 cGy (165 cGy BID, total 8 fractions, days -4 to -1)

Day	Preparative Regimen		Supportive Care/Other
-8	Fludarabine 25 mg/m^2 IV over 30 minutes		
-7	Fludarabine 25 mg/m^2 IV over 30 minutes Cyclophosphamide 60 mg/kg IV		
-6	Fludarabine 25 mg/m^2 IV over 30 minutes Cyclophosphamide 60 mg/kg IV		
-5	Rest		
-4	TBI 165 cGy twice daily		
-3	TBI 165 cGy twice daily		Begin CSA (as per Section 9.3A)
-2	TBI 165 cGy twice daily		
-1	TBI 165 cGy twice daily		
0	**EXPERIMENTAL ARM** **UCBT**: Unmanipulated unit(s) (umbilical cord blood #1 and/or #2), followed by	**STANDARD OF CARE ARM**	Begin MMF (as per Section 9.3B)

	infusion of ex vivo expanded cell product at a minimum of 4 hours after **completion*** of last unmanipulated unit	**UCBT**: Unmanipulated unit(s) (umbilical cord blood #1 and/or #2)	
+1			Begin G-CSF (as per Section 9.4A)

* See 9.6B.3

A. Fludarabine: 25 mg/m^2/day IV over 30 minutes x 3 days (days -8 to -6); total dose 75 mg/m^2. For patients > 120% of ideal weight, BSA will be calculated using adjusted weight.

B. Cyclophosphamide
   1. Cyclophosphamide: 60 mg/kg/Day IV x 2 days (Days -7 and -6); total dose 120mg/kg.
   2. Preparation, administration and monitoring will be according to Institutional Guidelines. If patient's actual weight is >100% of IBW, adjusted body weight should be used for calculating initial doses based on per kilogram weight, per Institutional Guidelines. MESNA will be given for bladder prophylaxis according to Institutional Guidelines. Continuous bladder irrigation is an alternative for bladder prophylaxis at the attending physician's discretion.
   3. Cyclophosphamide administration and hydration guidelines for outside centers will be reviewed and approved by FHCRC PI if varying significantly from the above. Monitoring for toxicities will be according to Institutional Guidelines.

C. Total Body Irradiation**:** TBI 165 cGy will be given twice daily for a total dose of 1320 cGy (days -4 to –1).

REGIMEN B: Middle Intensity TBI regimen (included ages: 6 months through 65 years old)

Fludarabine total 150 mg/m^2 (30mg/m^2 IV x 5 days, days -6 to -2), Cyclophosphamide total 50 mg/kg IV x 1 day, day - 6), Thiotepa total 10 mg/kg (5 mg/kg/day x 2 days, -5 to -40), TBI total 400 cGy (200 cGy/day x 2 days, -2 to -1)

Day	Preparative Regimen		Supportive Care/Other
-6	Fludarabine 30 mg/m^2 IV over 30-60 minutes Cyclophosphamide 50 mg/kg IV		
-5	Fludarabine 30 mg/m^2 IV over 30-60 minutes Thiotepa 5 mg/kg IV over 4 hours		
-4	Fludarabine 30 mg/m^2 IV over 30-60 minutes Thiotepa 5 mg/kg IV over 4 hours		
-3	Fludarabine 30 mg/m^2 IV over 30-60 minutes		Begin CSA (as per Section 9.3A)
-2	Fludarabine 30 mg/m^2 IV over 30 minutes TBI 200 cGy once daily		
-1	TBI 200 cGy once daily		
0	**EXPERIMENTAL ARM** **UCBT**: Unmanipulated unit(s) (umbilical cord blood #1 and/or #2), followed by infusion of ex vivo expanded cell product at a minimum of 4 hours after **completion*** of last unmanipulated unit	**STANDARD OF CARE ARM** **UCBT**: Unmanipulated unit(s) (umbilical cord blood #1 and/or #2)	Begin MMF (as per Section 9.3B)
+1			Begin G-CSF (as per Section 9.4A)

* See 9.6B.3

A. Fludarabine: 30 mg/m^2/day IV over 30 minutes x 5 days (days -6 to -2); total dose 150 mg/m^2. For patients > 120% of ideal weight, BSA will be calculated using adjusted weight

B. Thiotepa: 5 mg/kg IV x 2 days (days -5 to -4); total dose 10 mg/kg. For subjects > 125% of ideal weight, BSA will be calculated using adjusted weight.

C. Cyclophosphamide:
   a. 50 mg/kg IV x1 day (day -6)
   b. Preparation, administration and monitoring will be according to Institutional Guidelines. If patient's actual weight is >100% of IBW, adjusted body weight should be used for calculating initial doses based on per kilogram weight, per Institutional Guidelines. MESNA will be given for bladder prophylaxis according to Institutional Guidelines. Continuous bladder irrigation is an alternative for bladder prophylaxis at the attending physician's discretion
   c. Cyclophosphamide administration and hydration guidelines for outside centers can be according to Institutional Guidelines. Monitoring for toxicities will be according to Institutional Guidelines

D. Total Body Irradiation**:** TBI 200 cGy will be given once daily for a total dose of 400 cGy (days -2 to –1)

9.3 Immunosuppressive Therapies

All patients will receive GVHD prophylaxis with 2 drugs as follows:

A. Cyclosporine (CSA)
   1. Patients will receive cyclosporine (CSA) therapy beginning Day -3 maintaining a level of >200 ng/mL. For adults the initial dose will be 2.5 mg/kg IV over 1 hour every 12 hours. For children < 40 kg the initial dose will be 2.5 mg/kg IV over 1 hour every 8 hours. For outside centers, initial CSA administration can be according to Institutional Guidelines.
   2. Dose adjustments will be made on the basis of toxicity and CSA levels with a targeted trough level of 200-400ng/mL. Once the patient can tolerate oral medications and has a normal gastro-intestinal transit time, CSA will be converted to an oral form. Refer to Institutional Guidelines for conversion from IV to PO dosing, CSA dosing will be monitored and altered as clinically appropriate
   3. Initial cyclosporine dose is calculated using actual body weight except for those patients who are greater than 100% ideal body weight in which case calculation of dose using adjusted body weight is recommended.
   4. Patients will receive CSA until Day +100. If there is no GVHD, the dose will be tapered 10% per week beginning on Day +101, to discontinue **no sooner than** 6 months post transplant.

B. Mycophenolate mofetil (MMF)
   1. All patients will begin mycophenolate mofetil (MMF) on Day 0, starting approximately 4 - 6 hours after infusion of the cord blood unit(s) is completed OR after infusion of the expanded product is completed for patients randomized to this arm. All patients will receive MMF at the dose of 15 mg/kg (based on adjusted weight) every 8 hours with a maximum of 1 gram/dose. If actual body weight is < ideal body weight, calculation based on actual weight is allowed. Rounding of the dose to the nearest 250 mg capsule size is also allowed.
   2. Use IV route between days 0 and +7; then, if tolerated, may change to PO beginning on day +8 three times a day.
   3. All patients will remain on three times a day as permitted after day 7 for a minimum of 30 days. At day +30 **or** 7 days after engraftment (defined as 1st day of 2 consecutive days of absolute neutrophil count [ANC] $\geq 0.5 \times 10^9$/L), whichever day is later, **if there is no evidence of acute GVHD and donor CD3 engraftment is at least 50% from one donor**, taper MMF to BID. At day +45 (or 15 days after engraftment if engraftment occurred > day +30), if there continues to be no evidence of acute GVHD and donor engraftment has been achieved (defined as <10% host in CD3, CD33 and CD56), taper MMF over the next two to three weeks.
   4. If there is no donor engraftment, do not stop MMF. If there is no evidence of donor engraftment on the day +28 bone marrow biopsy, notify the PI, Filippo Milano (pager: 206-314-1037).

9.4 Growth Factor

A. All patients will receive G-CSF 5 mcg/kg/day IV (pediatric patients) or SC (adult patients) (dose rounded to vial size) based on the actual body weight beginning on Day +1 after UCB infusion. G-CSF will be administered daily until the ANC exceeds 2.5 x 10^9/L for three consecutive days and then discontinued. If the ANC decreases to ≤1.0 x 10^9/L, G-CSF will be reinstituted and titrated to maintain an ANC >1.0 x 10^9/L.

9.5 Supportive Care

A. Patients will receive transfusions, infection prophylaxis (bacterial, fungal, viral), and other therapy (including GVHD) according to Institutional Guidelines.

9.6 Cord Blood Infusion (FHCRC - see also Protocol Specific Infusion Orders)

A. Infusion of unmanipulated (freshly thawed) cord blood unit(s)

1. The unmanipulated (freshly thawed) cord blood unit(s) will be infused first.
2. Procedures for requesting, receiving and characterizing the cord blood unit(s) for infusion will be according to Institutional Guidelines.
3. Pre-infusion hydration should be performed per institutional guidelines.
4. Procedures for infusion of the cord blood unit(s) will be according to Institutional Guidelines. See Appendix B for FHCRC Standard Practice Guideline for Infusion of Cryopreserved Cord Blood for procedures at FHCRC and suggested guidelines for Participating Centers.
5. If the patient is a recipient of a double cord blood graft, the two unmanipulated units should be given consecutively with no wait between infusion of the units. **However, infusion of the second unit will not begin until any acute toxicities from the first unit have been controlled.** The start and stop time of each unit should be recorded on the infusion record.
6. **FHCRC:** If the cord blood unit(s) fail to pass inspection or if there is insufficient information to verify the cell product for the patient, notify the Cellular Therapy Lab ((206) 606-1200) and the PI (Filippo Milano, (206) 667-5925, pager (206) 314-1037 immediately).

B. Infusion of expanded, cryopreserved cord blood progenitor cells

1. Dosage and selection of expanded product: See Section 6.2 B
2. **FHCRC patients:** please see protocol specific infusion orders. Infuse product as provided by Cellular Therapy.
3. Infusion time of ex vivo expanded cell product is ideally approximately 4 hours after **completion** of infusion of last unmanipulated unit. Variation is allowable for the infusion time of ex vivo expanded cell product, provided that any toxicities related to infusion of the last unmanipulated product have resolved. The acceptable window of infusion time is 3 hours 30 minutes to 24 hours following completion of the last unmanipulated unit.

   The patient should be premedicated with diphenhydramine and acetaminophen. Additional diphenhydramine, epinephrine and hydrocortisone should be available at the bedside for emergency if necessary. If diphenhydramine and acetaminophen have been previously administered as premedication for the unmanipulated unit(s), repeated dosing is at provider discretion.
4. Oxygen with nasal prongs for standby use should be present in the room.
5. Filtration: Filter through a blood component administration filter set by gravity. If institutional guidelines include pump infusion use, please infuse by syringe pump only (no roller set infusions).
6. The goal rate for infusion of the expanded cells is no longer than 30 minutes, as clinically possible. Infuse at a rate of 3–5 ml/min for the first 4 minutes. If tolerated, increase rate to "wide open" as tolerated. No medications or fluids should be given piggyback through the catheter that is being used for the expanded cell infusion.
7. Monitoring of immediate toxicities: See Section 11.3.

9.7 CNS Prophylaxis and Testicular Irradiation

A. All patients with ALL will undergo a diagnostic lumbar puncture during the pre-admission work-up following institutional guidelines for diagnostic tap alone or with prophylactic therapy. AML patients previously demonstrated to be CNS negative prior to pre-transplant work-up may follow institutional guidelines for work-up of potential CNS disease, however it is strongly recommended that all AML patients undergo a diagnostic lumbar puncture during pre-transplant evaluation to rule out CNS involvement. For patients with CML and MDS, lumbar puncture and prophylactic therapy are performed per institutional guidelines.

B. No dose of intrathecal therapy is to be given less than 72 hours before marrow/stem cell infusion. Beginning on day 32 post transplant, patients will receive intrathecal (IT) Methotrexate as indicated, per institutional guidelines.

C. The CNS prophylaxis regimen may be modified by the attending physician in consultation with the clinical coordinator as clinically indicated (i.e., IT methotrexate can be omitted in patients with a low risk of CNS relapse or in patients who have a high risk of leukoencephalopathy).

D. FHCRC: Testicular irradiation will be performed per standard practice. Outside centers: testicular irradiation will be performed as possible and per institutional guidelines.

9.8 Management of Pre-Engraftment Immune Reactions

A. A well-recognized clinical entity consisting of skin rash, fever, and, often, loose stools and respiratory distress has been noted to occur prior to engraftment among cord blood patients, generally between Days +7 and +21. This clinical syndrome likely involves cytokine activation, and though clinically similar to acute or hyperacute graft versus host disease, it appears to be a distinct entity, "pre-engraftment syndrome." This syndrome is often controlled with brief steroid bursts, thus avoiding a commitment to extended steroid exposure. Patients should be monitored carefully for this syndrome.

If patients have moderate to severe symptoms as described above and alternative etiologies (i.e., infection) have been excluded or are being appropriately evaluated, recommendations for management are:

1. For patients not on steroid therapy when the syndrome occurs: methylprednisolone should be given at 1 mg/kg IV q day for three days. If symptoms have abated, steroids should be stopped. If symptoms persist, 1 mg/kg methylprednisolone can be continued through six days then stopped if symptoms have abated. If symptoms persist for more than six days, the patient should be considered to have acute/hyperacute GVHD and should be treated with prolonged steroids as deemed appropriate.

2. For patients already on steroids for other reasons when the syndrome occurs: methylprednisolone should be given at a dose of 3-5 mg/kg IV (max dose 500 mg) q 12 hours x 48 hours, followed by a rapid taper to 1 mg/kg IV q 12 hours. Patients should be weaned after response as tolerated.

10.0 EVALUATION *(in addition to procedures and evaluations listed below, additional clinical evaluations as directed by the clinical team may be captured for research purposes)*

10.1 Pre-Transplant Evaluation

FHCRC Patients: Refer to FHCRC/SCCA Standard Practice Manual for Pre-Transplant Evaluation Guidelines for Allogeneic Transplant. **(results of tests and/or procedures conducted as per standard of care for pre-transplant workups may be used for eligibility determination if conducted within an appropriate window prior to screening)**

Fred Hutch and Outside Patients: Upon approval of protocol version 3.12.19, please refer to Appendix G for an updated schedule of study evaluations for 1 and 2-year.

In addition to Standard Practice Guidelines complete the following protocol specific requirements:

A. Bone marrow aspirate (and biopsy as clinically indicated), CBC with differential, comprehensive metabolic panel, and creatinine clearance **within 30 days of start of conditioning.**
B. MUGA or echocardiography with measurement of the left ventricular ejection fraction (LVEF) or left ventricular shortening fraction.
C. Chest CT without contrast to exclude occult infection prior to transplant **within 30 days of start of conditioning.**
D. Pulmonary function tests; pediatric pre-transplant evaluation qualification: see section 5.1.3D
E. Viral screening including CMV PCR
F. DNA specimen from cord blood unit(s) for chimerism studies (FHCRC patients: submitted to CIL)
G. Panel Reactive Antibody (PRA) per Institutional Guidelines
H. Pregnancy test per Institutional Guidelines in females of childbearing potential.

10.2 Evaluation on the day of cord blood infusions (Day 0)

A. Physical exam and review of systems by provider
B. Weight
C. CBC with differential
D. Renal and hepatic function: NA, K, CL, CO2, GLU, BUN, CRE, CA, P, ALB, MG, ALT, AST, ALK, BILIT/D, TP, ALB, LDH
E. Complete urinalysis only on patients receiving the expanded cord blood progenitor cell product

10.3 Evaluation during infusion of Ex Vivo Expanded Cord Blood Progenitor Cell Product

A. Patient must be monitored during infusion as per institutional guidelines.
B. For any changes in cardiac or respiratory status, notify physician.
C. Obtain and record vital signs including temperature, BP, HR, respirations,O2 saturation at the following approximate time points:

Pre-infusion	1 hour after the start of infusion
15 minutes after the start of infusion	2 hours after the start of infusion
30 minutes after the start of infusion	4 hours after the start of infusion
45 minutes after the start of infusion	24 hours after the start of infusion

D. Please also refer to Section 11.3B

10.4 Evaluation approximately 24 hours post completion of infusion of expanded cord blood progenitor product

A. CBC with differential
B. Renal and hepatic function: NA, K, CL, CO2, GLU, BUN, CRE, CA, P, ALB, MG, ALT, AST, ALK, BILIT/D, TP, ALB, LDH
C. Complete urinalysis

10.5 Patient Evaluations during Therapy until Engraftment (Or Through Day 30, Whichever Occurs First)

A. Physical exam, interval history and vital signs daily while in the hospital. After discharge, vital signs twice weekly until engraftment.
B. Complete blood count with leukocyte differential daily (+/- 4 hour window) until the absolute neutrophil count (ANC) $\geq 5x10^8$/L for 2 consecutive measurements. For outside centers, complete blood count with leukocyte differential requirement can be according to Institutional Guidelines.
C. Serum electrolytes, BUN, creatinine, glucose every other day at a minimum until engrafted.
D. Hepatic function panel at least three times weekly or as clinically indicated.
E. CMV monitoring per Institutional Guidelines.
F. Urinalysis as clinically indicated.
G. Chest radiographs as clinically indicated.
H. Peripheral blood donor chimerism. See Section 10.7 for details.

I. Bone marrow aspirate (+/- biopsy, as clinically indicated) on Day 28 for disease restaging (morphology, immunophenotyping, cytogenetics and molecular studies as indicated) and assessment of donor engraftment. FHCRC patients: bone marrow specimen, *unsorted*, to CIL for chimerism studies.
J. Quantitative immunoglobulin levels (IgG, IgA, IgM) at Day 28
K. As possible, total T lymphocytes and subset enumeration at Day 28
L. Panel Reactive Antibody (PRA) upon count recovery on patients who received the expanded cells. **Please check with research staff (PI or Research Nurse (Nancy Anderson) or Research Coordinator (Denise Ziegler)). Research staff will notify clinical staff to initiate the orders. PRAs will be charged to research budget.**
M. GVHD evaluation (Appendix C) weekly or as clinically indicated
N. Other tests (e.g., x-rays and tumor markers) as clinically appropriate for assessment of underlying malignancy on Day 28
O. See also Section 11.3B

10.6 Patient Evaluations from Engraftment through Day 100, 6 Months, 1 Year and 2 Years

A. Physical examinations at least weekly until discharge, then as clinically indicated during first 100 days, 6 months, 1 year and 2 years
B. Vital signs twice weekly until discharge
C. Karnofsky/ECOG/Lansky performance status (Appendix A) once between Day 80 and Day 100, 6 months, 1 year and 2 years
D. Complete blood count with leukocyte differential at least weekly and/or as clinically indicated through Day 100 or until discharge then at 6 months, 1 year and 2 years
E. Serum electrolytes, BUN, creatinine, glucose twice weekly or as clinically indicated through day +100
F. Hepatic function panel at least weekly through Day 100 or until discharge and then as clinically indicated
G. CMV monitoring per Institutional Guidelines
H. Chest radiographs, EKG and pulmonary function tests as clinically indicated
I. Bone marrow aspirate (+/- biopsy as clinically indicated) on Day 80 and 1 year for assessment of donor engraftment and disease restaging. FHCRC patients: bone marrow aspirate submitted, *unsorted*, to CIL for chimerism studies.
J. Peripheral blood for chimerism studies. See Section 10.7 for details.
K. Quantitative immunoglobulin levels (IgG, IgA, IgM) at Day 56, 80, 6 months, 1 year and 2 years
L. As possible, total T lymphocytes and subset enumeration on Day 56, 100, 6 months, 1 year and 2 years
M. GVHD evaluation (Appendix C) weekly and/or as clinically indicated through Day 100 (or longer if clinically indicated), then at 6 months, 1 year and 2 years
N. Autopsy report, if available, if death occurs before the 2 year follow-up
O. See also Section 11.3B

10.7 Evaluation of In Vivo Persistence of Expanded and Unmanipulated Cord Blood Cells: Chimerism Studies/UCB Engraftment Evaluation

Chimerism studies will be performed as outlined below. Subsequently, if the patient's peripheral blood counts drop after an initial recovery, the peripheral blood and bone marrow should again be evaluated unless a cause has been determined (e.g., use of Ganciclovir for treatment of CMV). Patients diagnosed with graft failure as defined in section 13.0 must be reported to the Principal Investigator (Filippo Milano, 206-667-5925/pgr 206-314-1037).

A. Bone marrow aspirate (unsorted) for chimerism studies at day +28, +80 and 1 year. Day +80 may be performed on Day +100 at outside centers as per institutional guidelines. After 1 year, bone marrow chimerism is to be performed as clinically indicated. Send to Clinical Immunogenetics Laboratory.
B. Bone marrow aspirate for morphology and immunophenotyping by flow cytometry at day +28, +80 and 1 year. Day +80 may be performed on Day +100 at outside centers as per institutional guidelines. After 1 year, bone marrow evaluation is to be performed as clinically indicated.

C. Peripheral blood donor chimerism: Day +7, +14 and +21*
Peripheral blood is to be collected and cell sorted for $CD3^+$, $CD33^+$, $CD14^+$, and $CD56^+$, as possible at outside centers) and donor/host chimerism determined.
Peripheral blood donor chimerism: Day +28**, +42*, +56, +80, +180, 1 and 2 years:
Peripheral blood is to be collected and cell sorted for $CD3^+$, $CD33^+$, $CD14^+$, $CD19^+$ (or 20^+) and $CD56^+$ (as possible at outside centers) and donor/host chimerism determined. Day +80 may be performed at Day +100 at outside centers as per institutional guidelines. After 1 year, peripheral blood chimerism is to be sorted for $CD3^+$, $CD33^+$, and $CD56^+$ only, unless otherwise clinically indicated.

*Draw peripheral blood for chimerism at Day +21 and Day +42 **only if** previous chimerism time point did not show at least 95% donor engraftment from a single cord blood unit.
**Day +28 chimerism may be skipped IF the patient had Day 21 chimerisms and it showed at least 95% donor engraftment from a single cord blood unit.

10.8 Residual/Recurrent Disease Evaluation

A. Patients will be evaluated routinely for evidence of recurrent malignancy as per Institutional Guidelines on day +28 and day +80. If at any time the attending physician suspects recurrent disease, additional analyses will be performed as clinically indicated.

10.9 Immune Reconstitution

Clinical Studies: As possible, quantitative immunoglobulin levels (IgG, IgA, IgM) will be assessed at pre-conditioning, Day 28, 56, 80 or 100 (per institutional practice), 6 months, 1 year and 2 years. As possible, total T lymphocytes and subset enumeration (lymphocyte panel) will be performed at Day 28, 56, 100, 6 months, 1 year and 2 years.

Research Studies: Upon approval of this protocol modification, all research blood collections will discontinue. Adaptive Biotechnologies will continue to conduct TCR studies (immunophenotypic evaluation of immune reconstitution) using previously collected de-identified samples and data.

10.10 Study Evaluation Windows

The target dates for post-transplant study evaluations are outlined in the table below. In certain clinical circumstances (e.g., relapse, clinical status or terminal illness) study tests may be omitted at the physician's or PI's discretion.

Evaluation Timepoint Post Transplant	Window
24 hrs post infusion of expanded cell product	± 2 hours
Day +7	± 2 days
Day +14	± 3 days
Day +21	± 3 days
Day +28	± 3 days
Day +42	± 7 days
Day +56	± 7 days
Day +80	± 7 days
Day +100	± 7 days
Day +180	± 30 days
1 year	*
2 year	*

* Every effort will be made to complete the 1 year and 2 year evaluations as close to these dates as possible, taking into consideration patient's circumstances at these time points.

11.0 DRUGS, IRRADIATION AND CORD BLOOD INFUSION: TOXICITIES AND COMPLICATIONS

11.1 Cyclophosphamide and Other Conditioning Agents

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of 100	**Uncommon** Occurs in <5 people out of 100
Nausea/vomiting Mucositis Sterility Severe suppression of blood counts Diarrhea Fluid weight gain or edema Alopecia	Hemorrhagic cystitis	Cardiomyopathy Skin rash SIADH (Syndrome of Inappropriate Anti-diuretic Hormone)

Fludarabine

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of 100	**Uncommon** Occurs in <5 people out of 100
Severe suppression of blood counts Diarrhea Anorexia Mucositis Nausea/vomiting Stomatitis Osteoporosis Dysuria	Chills Fever GI bleeding Peripheral edema	Neurotoxicity Agitation and confusion Blurred vision Peripheral neuropathy Hearing loss Headache Cerebellar syndrome Blindness Coma Weakness Depression Insomnia Hemorrhagic cystitis (except in FA) Abnormal renal function test Autoimmune hemolytic anemia Deep venous thrombosis Aneurysms Pruritic skin rash Abnormal liver function/Liver failure Constipation Transient ischemic attack Dysphagia Myalgia Arthralgia Renal failure

Thiotepa

Likely (Over 10%)	Less Likely (1-10%)	Rare (Less than 1%)
• Low white blood cell count with an increased risk of infection (from bacteria, fungi or viruses) • Low platelet count with increased risk of bleeding • Anemia • Nausea/vomiting • Diarrhea • Anorexia (loss of appetite) • Mouth ulcers • Sores in mouth or on lips • Missing or stopping of menstrual periods in women	• Skin rash • Change in skin coloring • Fatigue, weakness • Dizziness • Headache • Permanent sterility (inability to have children)	• Allergic reactions during infusion (fever, chills, itching, hives, flushing, rash, shortness of breath, wheezing, chest tightness, muscle stiffening) • Confusion • Seizures • Liver damage • Secondary cancers

11.2 Total Body Irradiation

See Institutional Guidelines for recommendations and technical parameters for administration, toxicity and complications of TBI by linear accelerator.

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of 100	**Uncommon** Occurs in <5 people out of 100
Nausea and vomiting Diarrhea Cataracts Sterility Endocrinopathies Growth failure Intestinal cramps Mucositis	Parotitis Interstitial Pneumonitis Generalized mild erythema Veno-occlusive disease	Dysphagia Vertebral deformities Nephropathy

11.3 Infusion of Unmanipulated and Expanded Cord Blood Units

A. Unmanipulated Cord Blood Units

Potential toxicities: Although the cord blood cells are thawed per standard institutional procedures prior to infusion, potential toxicities associated with the infusion include DMSO toxicity and side effects from red cells. Allergic reactions to the thawing diluent (especially dextran) have also been reported. DMSO toxicity and side effect of red cells may include changes in heart rate, rhythm or function, changes in blood pressure, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, headache, allergic reaction, presence of DMSO taste and odor, hemoglobinuria, and acute renal failure.

B. Expanded Cells

Please refer to Section 9.6 for infusion guidelines

1. Immediate toxicities: Immediate toxicities (those occurring either during or within the first 24 hours following the infusion of the expanded cord blood unit) might occur. These symptoms could include fever, chills, fatigue, dyspnea, chest tightness or myalgias; alteration in vital signs such as hypotension, tachycardia, tachypnea, or hypoxemia; and skin changes such as erythema, urticaria, or other rash. Such symptoms will be managed by acetaminophen, diphenhydramine, intravenous fluids

and supplemental oxygen. Severe toxicity, including but not limited to, fever (>38.5^{0}C), hypotension (adults: systolic BP <90 mm Hg and ≥ 20 mm Hg below baseline), tachycardia (adults: HR >130), tachypnea (adults: RR >32) or hypoxemia (arterial O2 saturation <90%) will be evaluated and managed by the clinical team. The infusion may be terminated by the team if deemed clinically necessary, followed by supportive medical care. Termination of the infusion for pediatric patients will be at the treating physician's discretion. If the patient does not respond adequately to supportive care alone, methlyprednisolone will be administered at 2mg/kg IV every 12 hours for 2 total doses.

2. Delayed toxicities: It is possible that infusion of the expanded cells may lead to graft failure or increased rates of acute or chronic GVHD. As such, there are stopping rules in place for these possible delayed toxicities (Section 16). Patients with potential graft failure will be treated per institutional guidelines. Patients with acute or chronic GVHD will be treated as directed by their primary clinicians and/or per recommendations of GVHD specialists as per institutional practice.

11.4 Granulocyte Colony Stimulating Factor (GCSF)

Bone pain	Insomnia
Headaches	Dyspnea
Body aches	Rash
Fatigue	Edema
Nausea/vomiting	

11.5 Cyclosporine

Nephrotoxcity	Thrombotic thrombocytopenic purpura
Seizures	Electrolyte imbalances
Hypertension	Paresthesias/neuropathy
Hirsutism	Gingival hyperplasia
Increased risk of relapse	Increased risk of opportunistic infection

11.6 Mycophenolate Mofetil (MMF)

MMF is supplied in 250 mg hard gelatin capsules or intravenous formula. Capsules can be stored at room temperature.

A. Precautions: Mycophenolate mofetil has not extensively been previously studied in patients after marrow transplantation. Previous clinical studies in patients after renal allografting suggested that the principal adverse reactions associated with the administration of MMF include diarrhea, leukopenia, sepsis, vomiting and a higher incidence of certain viral infections (CMV, VZV, Herpes Simplex). Patients will be monitored for the development of these complications.

B. Adverse Events: MMF may be associated with vomiting and diarrhea, decline in hematocrit and white blood cell count, and infection. In the setting of marrow transplantation, several etiologic factors may contribute to alterations in G.I. and hematologic parameters. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the PI. Dose adjustments are described in Section 9.3.

Potential Toxicities Associated with Mycophenolate Mofetil (MMF)

Pancytopenia	Hypertension
Headache	Dizziness
Insomnia	Hyperglycemia
Electrolyte imbalances	Rash
Leg cramps/bone pain	Nausea/diarrhea
Spontaneous abortion	Birth defects

Progressive multifocal leukoencephalopathy	

12.0 GUIDELINES FOR ADVERSE EVENT REPORTING

12.1 Adverse Event Reporting/Institutional Policy

The following guidelines are the minimum Cancer Consortium IRB adverse event (AE) reporting guidelines. Protocol-specific additional reporting requirements for adverse events are addressed in Section 12.2.

In accordance with institutional policy, all adverse events which in the opinion of the principal investigator are unexpected and related or possibly related to the research and serious or suggest that the research places research participants or others at greater risk of physical or psychological harm than was previously known or recognized are to be reported to the IRB within 10 calendar days of learning of the problem.

Definitions:

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

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction.

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 any predisposing risk factor profile for the adverse event.

Serious Adverse Event (SAE) – Any adverse event occurring 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 (see section 12.2)
- a persistent or significant disability/incapacity
- a congenital anomaly
- requires intervention to prevent permanent impairment of damage

Attribution - The following are definitions for determining whether an adverse event is related to a medical product, treatment or procedure:

- An adverse event is "**related or possibly related to the research procedures**" 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.

12.2 Protocol –Specific Collection, Grading and Reporting of Adverse Events

Patients enrolled in this study are receiving myeloablative pre-transplant treatments and other transplant-related procedures that are generally associated with high rates of "expected" adverse events. Refer to Appendix D for a list of potential adverse events associated or expected with hematopoietic cell transplantation. Toxicities will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. The scale in its entirety can be found at: http://www.acrin.org/Portals/0/Administration/Regulatory/CTCAE_4.02_2009-09-15_QuickReference_5x7.pdf

All patients undergoing allogeneic transplantation using any investigational or standard myeloablative approach are expected to experience multiple grade 1-3 adverse events. It is expected that local PIs will be reviewing these AEs as documented by signing off on CRFs. Grade <4 hematologic toxicity is expected and will be recorded as time to engraftment. All AEs/SAEs will be followed until resolution or until the PI attributes the AE/SAEs to a cause other than the study drug or assess them as chronic or stable and captured in protocol specific case report forms:

- Non-hematologic adverse events of ≥ grade 3
- Grade ≥4 blood and lymphatic system disorder-related adverse events occurring between Day 43 and Day 100
- Serious adverse events as defined above

The following events are not identified as AEs in this study:

- Disease progression or relapse. However, clinical events associated with progression/relapse may be reportable as AEs.
- Hospitalization for the purpose of facilitating stem cell transplant, for protocol-scheduled procedures, blood product transfusions, or for social reasons (i.e., awaiting transport home) will not be considered a serious adverse event. Previously, hospitalization occurring at any time pre- or post-transplant were considered a SAE only if it was unexpected or the duration of the hospital stay was unexpected per the definition of "unexpected adverse event" above. As of this protocol amendment, hospitalizations occurring at any time pre- or post-transplant are considered an SAE regardless of meeting the "unexpected adverse event" definition above and will be collected and reported retroactively and prospectively for all study patients. Retroactive collection of expected hospitalizations will not follow the protocol SAE reporting timelines.
- Medical or surgical procedures in and of themselves, including those that require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.
- All patients undergoing hematopoietic stem cell transplant are expected to have ≤ Grade 4 pancytopenia as an intended therapeutic effect. These hematologic AEs will therefore be tracked and recorded between day 0 and 43 only as time to recovery of blood counts/engraftment.
- Abnormal laboratory values will be identified and recorded as AEs only if clinical intervention is required as a result.

When a grade 3 adverse event increases in severity to grade 4 or above, the event will be captured at its highest grade. If a patient experiences relapse or graft failure and goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death.

After day 100 patients will be tracked for progression and survival as well as secondary endpoints.

Graft versus Host Disease assessment, performed by the clinical team as part of routine care, will be reviewed once weekly through Day +100. After Day +100, GVHD data will be captured at Day +180, 1 year, then once yearly through 2 years after Day 0. GVHD data is captured in a GVHD-specific CRF.

Relapse, graft failure and death data will be captured as they occur in CRFs specific to those events. If a patient experiences relapse or graft failure and goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death.

Coordinating Center IRB reporting

In accordance with FHCRC institutional policy, AEs from any participating site that affect the risk assessment of the trial as a whole (indicates that the research places research participants or others at a greater risk of harm) and those occurring at FHCRC as a participating site which in the opinion of the FHCRC principal investigator meet all three of the following criteria will be reported to the Coordinating Center IRB within 10 calendar days of learning of the problem:

Expedited reporting criteria:

A. unexpected
B. related or possibly related to the research
C. serious or suggests that the research places research participants or others at greater risk of physical or psychological harm than was previously known or recognized

In addition, severe infusion reactions related to the expanded cellular product requiring termination of the infusion will be reported to the Coordinating Center IRB within 10 calendar days of learning of the problem.

All other SAEs and deaths, not meeting the expedited reporting criteria, will be reported to the IRB as part of the annual continuation review report to the IRB. The FHCRC principal investigator will comply with IRB Policy 1.1 on Reporting Obligations for Principal Investigators. The FHCRC PI will report all serious adverse events to the IND Sponsor (Nohla Therapeutics, Inc.) within the timeline described in the table below.

FDA reporting (IND Sponsor Responsibilities)

The IND Sponsor assumes responsibility for IND safety reporting to the FDA and participating investigators, in accordance with regulations under 21 CFR 312.32. Each SAE report received from the PI will be evaluated by the IND Sponsor who will assess the seriousness of the event, the expectedness of the event, and the relationship to participation in the study. For regulatory reporting purposes, the IND Sponsor will determine expectedness relating to the investigational agent using safety information specified in the investigator brochure and consent form. An event will be classified as related if either the investigator or the IND Sponsor determines that the event may be related to the investigational agent.

For determination of IND safety reporting, AE attribution will be assessed according to the suspected adverse reaction definition described in 21 CFR 312.32 as an AE for which there is a reasonable possibility that the investigational agent caused the adverse event where “reasonable possibility” means there is evidence to suggest a causal relationship between the investigational agent and the AE. Suspected adverse reactions that are serious, related, and unexpected will be reported to the FDA as an IND safety report, in accordance with regulations under 21 CFR 312.32.

IND safety reports will be submitted to the FDA by the IND Sponsor for serious, unexpected suspected expanded cell product adverse reactions within 15 calendar days of the IND Sponsor determining that the information requires reporting. The IND Sponsor will submit all unexpected fatal or life-threatening events associated with the expanded cell product to the FDA fax or phone within seven days of initial receipt of information from the PI. Safety reports will also be submitted for severe infusion reactions associated with the expanded cellular product requiring termination of the infusion. IND safety reports will be forwarded by the IND Sponsor to the Coordinating Center. The PI will distribute the IND safety reports to sites and submit IND safety reports to the FHCRC IRB, consistent with the IRB policy. *Participating site investigators will be responsible for local IRB submission of trial-wide safety reports.*

All SAEs must be reported to the IND Sponsor from the time of the investigational agent infusion until SAE duration as outlined in the protocol. SAEs that occur after the study-specific informed consent is signed but prior to the first dose of the investigational agent will be collected only if they are considered by the investigator to be causally related to the study required procedures. SAEs will be reported by the FHCRC PI or Research Nurse to the IND Sponsor according to the following timelines described in the table below:

Classification		Reporting Time	Reporting Action	FHCRC PI and Research Nurse Contact Information	IND Sponsor Contact Information
SAE	Fatal or life-threatening [1]	Within 24 hours of research team[2] awareness	Email notification to IND Sponsor (via completed SAE Reporting Form signed by PI or designated sub-Investigator) to FHCRC PI or Research Nurse	FHCRC PI email: fmilano@fredhutch.org Research Nurse email: nlanders@fredhutch.org	IND Sponsor email: safety@nohlatherapeutics.com
	All SAEs	Within 3 business days of research team[2] awareness	Submit completed IND SAE Reporting Form signed by PI or designated sub-Investigator to FHCRC PI or Research Nurse	FHCRC PI email: fmilano@fredhutch.org Research Nurse email: nlanders@fredhutch.org	IND Sponsor email: safety@nohlatherapeutics.com
Non-serious AE		Per CRF completion guidelines	Record on appropriate CRFs	N/A	N/A

[1] Includes severe infusion reactions associated with the expanded cellular product requiring termination of the infusion.
[2] Research team is defined as the individuals listed on the delegation of authority log. Physicians listed on the study's delegation of authority log as transplant service attending physician's delegated authority to administer informed consent will not be considered part of the research team unless additional responsibilities related to the conduct of the study have been delegated to them by the Principal Investigator.

NHLBI reporting (FHCRC only)

Any event or problem that is (1) unexpected, (2) possibly, probably, or definitely related to study participation, and (3) one of the following will be reported to the NHLBI Program Official within the noted timeframe: fatal, life-threatening, or serious (report within 7 days); suggests greater risk of harm to study participant(s) than was previously known or recognized (report within 30 days).

Participating Centers

Adverse events meeting expedited reporting criteria (see Coordinating Center IRB reporting section for criteria) must be reported to the Coordinating Center within 3 working days (Monday-Friday) of learning of the event and to local IRBs per institutional policy. The report must include the date, severity and duration of the event, the relationship to the study, the treatment given and eventual outcome. If the event is ongoing, follow-up information must be submitted as soon as the relevant information is available.

Adverse events that must be recorded per Section 12.2 that do not meet FHCRC expedited reporting criteria will be reported to the Coordinating Center at the regularly scheduled CRF intervals (see Section 14.0).

Participating centers must also report (1) any other unanticipated problems or events that place research participants or others at greater risk of harm (e.g. breach of confidentiality) or (2) instances of serious or continuing noncompliance with the IRB-approved protocol to the FHCRC Coordinating Center within 5 working days of the local PI learning of the event. If there is uncertainty regarding whether an event requires expedited reporting to the Coordinating Center, the event should be reported. The following noncompliance events must be reported to the Coordinating Center on an expedited basis whether or not the local PI considers them serious: enrolling a patient who does not meet eligibility criteria, failure to obtain informed consent for research procedures, and administering study drug/product at a dose that is not IRB-approved. A written report must be submitted to the FHCRC Coordinating Center with the following information: a description of the event, assessment of whether the event placed a participant or others at increased risk of harm, whether re-consenting has or will occur, whether changes to study documents are required, the plan of action to prevent similar occurrences in the future, and whether the event has been reported to the local IRB on an expedited basis. A case report form is provided for this purpose. Unanticipated problems that do not increase risk and minor noncompliance may be reported at the regular CRF timepoints. A link to FHCRC IRB policies, including those regarding unanticipated problems and noncompliance, is provided in Appendix E.

13.0 ASSESSMENT OF DISEASE RESPONSE

A. Primary Graft Failure: Patients will be considered primary graft failure/rejection provided they meet any criteria listed below:
   i. Absence of 3 consecutive days with neutrophils ≥500/ul combined with host CD3 peripheral blood chimerism ≥ 50% by day +42
   ii. Absence of 3 consecutive days with neutrophils ≥500/ul under any circumstances at day 55
   iii. Death after day 28 with neutrophil count <100/ul without any evidence of engraftment (< 5% donor CD3)
   iv. Primary autologous count recovery with < 5% donor CD3 peripheral blood chimerism at count recovery and without relapse

B. Secondary graft failure/rejection: Decline of neutrophil count to <500/ul with loss of donor chimerism after day 55.

C. Inadequate graft function: Patients who have met the definition of engraftment as defined above will be considered to have inadequate graft function if by day 80 they continue to require GCSF to maintain an ANC>1000 and/or they remain platelet transfusion dependent in the absence of GVHD or infectious complications (e.g., clinically significant infections/sepsis, BK viruria).

D. GVHD will be graded according to Appendix C.

E. Kinetics of Engraftment: the kinetics and durability of hematopoietic reconstitution will be assessed and the relative contribution to engraftment of the expanded cord blood product and the unmanipulated unit(s) will be determined by frequent peripheral blood donor chimerism assays (as in Section 10.6).

14.0 DATA AND SAFETY MONITORING PLAN

This is a multi-institution Phase 2 clinical trial that is monitored by the principal investigator (PI) and an independent Data and Safety and Monitoring Board (DSMB). The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the FHCRC Scientific Review Committee and Institutional Review Board. The PI will ensure that the Fred Hutch/UW Cancer Consortium Data and Safety Monitoring Plan (DSMP) is followed, that all data required for oversight of monitoring are accurately reported to the DSMB, that all adverse events are reported according to the protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported.

Under the provisions of the Fred Hutch/UW Cancer Consortium Data and Safety Monitoring Plan (DSMP), the Cancer Consortium Clinical Research Support Office provides monitoring for quality process and compliance by qualified monitors unaffiliated with the conduct of the study. Monitoring visits occur at specified intervals while accrual is ongoing. The scope of monitoring is specified in the Cancer Consortium DSMP:

http://www.cancerconsortium.org/content/dam/consortium/Resource-Documents/Data-Safety-Monitoring/Fred%20Hutch%20Consortium%20DSMP_Nov20_2015%20Final_Signed.pdf
Nohla Theraputics, as the IND Sponsor, will have access to participants' complete medical records, study treatment accountability records, and other source documents as needed to appropriately monitor the trial, in addition to, site regulatory files. Data from collaborating centers will be summarized and transmitted as paper case report forms (CRFs), then entered by Coordinating Center staff into the Fred Hutch research database. Case report forms (CRFs) must be submitted to the Fred Hutch coordinating center for the following time points: approximately day + 28 , +100, and +180 post transplant and at 1 year and 2 year follow up. CRFs are expected to be submitted as expeditiously as possible after the relevant time points (within 28 days). When possible, primary source documents regarding patient outcomes are collected from the collaborating centers with the case report forms to allow verification of protocol compliance, data accuracy and completeness and full and timely reporting of safety data.

This protocol has a dedicated independent DSMB responsible for monitoring patient safety, and assessing the safety and efficacy of the interventions during the trial. The DSMB meets at six-month intervals and all outcome data is reviewed including all adverse events. The DSMB determines whether the trial has met any stopping rules and reviews any patient safety problems potentially necessitating discontinuation of the trial. A report from the DSMB is submitted to the NHLBI Program Official, the FHCRC IRB and to the study coordinators/local PIs of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual.

15.0 DATA MANAGEMENT/CONFIDENTIALITY

Study data will be recorded in a password-protected research database. Case report forms are generated from the database for review by the principal investigator and study monitors. Clinical Statistics maintains a subject database at Fred Hutch to allow storage and retrieval of subject data collected from a wide variety of sources. The investigator will ensure that data collected conform to all established guidelines for coding, collection, key entry and verification. Each subject is assigned a unique patient number to assure subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files are scanned and stored in a secure database (OWL). OWL records are maintained by the Fred Hutch data abstraction staff. Access is restricted to personnel authorized by the Division of Clinical Research.

16.0 STATISTICAL CONSIDERATIONS

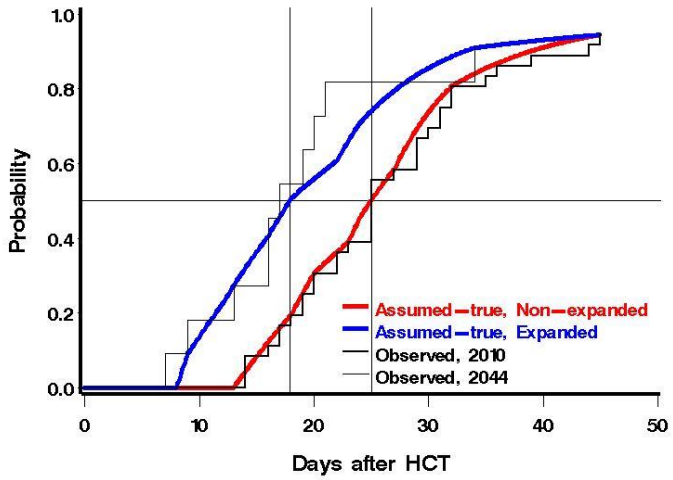
Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex / Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	15	18	33
Not Hispanic or Latino	64	63	127
Ethnic Category Total of All Subjects*	79	81	160
Racial Categories			
American Indian / Alaska Native	4	2	6
Asian	15	12	27
Native Hawaiian or Other Pacific Islander	1	4	5
Black or African American	8	7	15
White	51	56	107
Racial Categories: Total of All Subjects*	79	81	160

A Statistical Analysis Plan (SAP) has been developed for this protocol, and the analyses and concepts laid out in the SAP take precedence over anything listed in the protocol in the case where there are discrepancies.

The primary objective of this randomized phase II open-label, multi-center study is to compare the hazard of engraftment (defined as the first of 2 consecutive days in which ANC≥500) among recipients of single or double CBT without ex vivo expanded off-the-shelf CB progenitor cells to that among recipients of expanded off-the-shelf CB progenitor cells in patients with hematologic malignancies. Time to engraftment is associated with day-200 NRM and overall mortality in recipients of CBT (see preliminary data), and as such we view this as a reasonable endpoint for the current purposes, as opposed to NRM or OM, endpoints that would be considered for a primary endpoint in a subsequent Phase III trial should the current trial prove to be potentially efficacious, primarily with respect to engraftment. Secondary endpoints aimed at further assessment of economic and clinical benefit for the current trial include time to platelet recovery, duration of initial hospitalization, NRM, OS, GVHD, and severe infection. In exploratory analyses, other factors such as such as HLA-matching, cellular composition, cell dose and in vivo functional assays in an immunodeficient mouse model will also be examined in an effort to begin to understand factors that predict in vivo persistence and/or rapid engraftment of our off-the-shelf product infused into patients. In addition, an exploratory subgroup analysis based on conditioning regimen will be conducted, allowing us to examine separately the difference in outcome between the expanded arm compared to the non-expanded among patients who received high-dose TBI and among those who receive middle intensity TBI.

We propose to randomize 80 patients per arm based on the following considerations, and randomization will be stratified on the number of cord blood units using a permuted block design with random block sizes. Shown in the figure below is the observed time to engraftment for patients who have received non-expanded CB (via protocol 2010) at our Center. A piece-wise exponential curve was fit to these data, this fitted curve (in red) serving as the assumed-true time-to-engraftment distribution for patients who receive a single or double CBT without ex vivo expanded off-the-shelf CB progenitor cells. The median time to engraftment associated with this assumed-true distribution is 25 days (the observed median time to engraftment in this group is also 25 days). Shown in blue is the assumed-true time-to-engraftment distribution for the group to receive ex vivo expanded cells, the true median time being 17.9 days. We feel that this distribution is achievable, as it is consistent with what has been observed in Protocol 2044, where the median time to engraftment was 17 days.



We have chosen to use the log-rank test, as this not only considers when patients engraft, but time that patients have been followed without engraftment (primarily due to death without engraftment), and failure to account for the non-engrafters (particularly if the rate in the two arms appears to be different) could be misleading (for example, using the t-test among engrafters). We have also done extensive simulation studies that show the log-rank test and Gray's test (where deaths without engraftment are treated as competing-risk failures) have very similar power (with the log-rank test slightly higher) under a wide variety of scenarios. With 80 patients per arm and the above assumed-true time-to-engraftment distributions, we'll have approximately 87% power to observe a statistically significant difference (at the two-sided level of .05) in engraftment rates (power estimated from 5,000 simulations). As a secondary analysis of engraftment, we shall also compare groups using Gray's test. We shall also compare the risk of the competing risk (death without engraftment) between groups using both the log-rank and Gray's test. The log-rank test compares the cause-specific hazards of "failure", while Gray's test compares the cause-specific cumulative incidence function of "failure". Although we expect that patient and treatment characteristics will be well-balanced between study arms due to random allocation of patients, but in the event that there is some imbalance, we will carry out analyses comparing hazards

using Cox proportional hazards regression and competing risks hazard regression models adjusted for relevant factors.

An interim analysis for superiority will be conducted when 80 patients have been enrolled and followed sufficiently long to assess their time to engraftment. An O'Brien-Fleming boundary will be used at this interim analysis in order to maintain most of our type I error rate of .05 at the final analysis. In particular, regarding the primary endpoint of time to engraftment (ANC > 500), if there is a statistically significant difference in favor of the experimental regimen at the two-sided significance level of .0054, the boundary would be crossed at that interim analysis. The final analysis after 80 patients per arm are enrolled (should the trial run to completion) will be conducted at a significance level of .0492 (per the O'Brien-Fleming boundary). To enable the trial to provide more reliable benefit would occur only if the O'Brien-Fleming boundary also is crossed for the endpoint of overall survival, where full information on survival will be assumed to be 35 deaths.

Although not the primary objective of this study, we will continue to monitor for safety of infusion of non-HLA matched ex vivo expanded cryopreserved cord blood progenitors as adjunct therapy in the context of conventional single or double cord blood transplant. The endpoints to be used for this purpose include severe (Grades 3-4) acute GVHD, Grade $\geq$3 infusion toxicity and graft failure (see Section 13). It is not our objective to show that the rate of failure for these safety endpoints is less than that seen in conventional cord blood transplant with unmanipulated units, but rather that the rate is consistent with acceptable values. Towards this end, we have implemented stopping rules for each of these complications.

The study will be suspended pending further review if there is sufficient evidence to suggest that the true probability of severe GVHD exceeds 25%, the true probability of Grade $\geq$3 infusion toxicity exceeds 10%, or the true probability of graft failure exceeds 15% in the group of patients randomized to receive expanded cells. Sufficient evidence will be taken to be an observed failure rate whose lower one-sided 90% confidence limit exceeds the thresholds listed above (25% for GVHD, 10% for infusion toxicity, 15% for graft failure). The observed failure rates will be examined after every 10^{th} enrolled patient becomes evaluable. Operationally, these limits will be met if any of the following is observed.

Grade 3-4 acute GVHD: 5/10, 9/20, 12/30, 15/40, 17/50, 20/60, 23/70, 26/80, 29/90, 32/100, 34/110, 37/120, 40/130, 43/140, 45/150, 48/160
Grade $\geq$3 Infusion Toxicity: 3/10, 5/20, 6/30, 7/40, 9/50, 10/60, 11/70, 13/80, 14/90, 15/100, 16/110, 17/120, 18/130, 20/140, 21/150, 22/160
Graft Failure: 4/10, 6/20, 8/30, 10/40, 12/50, 14/60, 15/70, 17/80, 19/90, 21/100, 22/110, 24/120, 26/130, 27/140, 29/150, 31/160

The table below summarizes the probability of each of these limits being met (latter 3 columns of table) by 40,70,100 or 130 patients under a variety of assumed-true failure probabilities (2nd column of table). For example, the probability of suspending the trial due to excess GVHD at or before 40 patients are accrued is 0.95 if the true probability of GVHD is 0.40; the probability of suspending the trial due to excess graft failure at or before 70 patients is 0.97 if the true probability of graft failure is 0.30. Probabilities of suspension were estimated from 5,000 simulations.

Probability of Stopping from Listed Event				
Number Pts	True Prob of Failure	Grade 3-4 acute GVHD	Grade $\geq$3 Infusion Toxicity	Graft Failure
40	.20	.04		
70	.20	.05		
100	.20	.05		
130	.20	.06		
40	.40	.76		
70	.40	.95		
100	.40	.98		

130	.40	.99		
40	.05		.02	
70	.05		.02	
100	.05		.02	
130	.05		.02	
40	.20		.75	
70	.20		.90	
100	.20		.95	
130	.20		.99	
40	.10			.03
70	.10			.03
100	.10			.03
130	.10			.03
40	.30			.85
70	.30			.97
100	.30			.99
130	.30			1.00

If any of the above stopping rules are met at any time, the study will be suspended pending review by a DSMB for a recommendation regarding termination or continuation of the trial.

17.0 TERMINATION OF STUDY

The PI or IND Sponsor may terminate the study at any time. The IRB and FDA also have the authority to terminate the study should it be deemed necessary.

REFERENCES

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Appendix A - Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Lansky Play Performance Scale	
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
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 B

FHCRC Standard Practice Guideline: "Infusion - Cryopreserved Hematopoietic Progenitor Cells, Marrow, Apheresis, or Cord Blood (HPC, Marrow, HPC, Apheresis, HPC, Cord Blood), or Therapeutic Cells (TC, Apheresis)"

This document is provided as a suggested guideline for Participating Centers.

Cryopreserved
Infusion rev102611

Appendix C - GVHD Assessment

ACUTE GVHD GRADING SCALE

Severity of Individual Organ Involvement			
System	Severity		
Skin	☐	+1	maculopapular eruption involving less than 25% of the body surface
	☐	+2	maculopapular eruption involving 25-50% of the body surface
	☐	+3	generalized erythroderma
	☐	+4	generalized erythroderma with bullous formation + 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	☐	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	☐	+1	Nausea or vomiting or anorexia
	☐	+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

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

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

ACUTE GVHD ASSESSMENT

Staging by Individual Organ Involvement

SKIN: measured by rash first appearing generally between 10 and 70 days after transplant. (excludes rashes of known viral or other origin)

Stage	Description
1	Maculopapular rash <25% BSA
2	Maculopapular rash 25 – 50% BSA
3	Generalized erythroderma
4	Generalized erythroderma with bullous formation and desquamation

LIVER*: measured by total serum bilirubin

Stage	Description
1	2.0 – 2.9 mg/dL
2	3.0 – 5.9 mg/dL
3	6.0 – 14.9 mg/dL
4	$\geq$ 15.0 mg/dL

GUT:** includes only diarrhea occurring after Day +21

Score	Adult	Pediatric***
1	upper GI (anorexia, nausea, vomiting) with diarrhea of <1000 mL/day	upper GI (anorexia, nausea, vomiting) with diarrhea of <555 mL/m^2/day
2	1000 – 1499 mL/day diarrhea	556-833 mL/m^2/day diarrhea
3	$\geq$ 1500 mL/day diarrhea	>833 mL/m^2/day diarrhea
4	severe abdominal cramping, bleeding or ileus caused by GVHD	

* In cases where another cause of hyperbilirubinemia antedated the onset of rash, the liver score should be decreased by one stage.

** In cases where peak GI symptoms are exacerbated by a cause other than GVHD, the gut score should be decreased by one stage.

*** Pediatric patients <17 years of age

ACUTE GVHD ASSESSMENT

Overall Grade

The determination of an overall GVHD grade should be based on the organ stage, response to treatment and whether GVHD was a major cause of death.

Overall Grade	Organ Stage	Qualifying Conditions	Additional Qualifying Conditions
I	Stage 1 -2 skin	No liver or gut	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD.
II	Stage 3 skin or Stage 1 liver or Stage 1 gut	N/A	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD, but glucocorticoid treatment after the onset of GVHD was generally sufficient to control the disease.
III	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**without** GVHD as a major contributing cause of death	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD and that additional treatment after the onset of GVHD did not readily control the disease.
IV	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**with** GVHD as a major contributing cause of death	GVHD was resistant to both the prophylactic immunosuppressive regimen and any additional treatment after the onset of the disease.

References:

1. Leisenring, WM, Martin, PJ, Petersdorf, EW, et al. An acute graft-versus-host-disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens, Blood, 2006:108: 749-755.
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2603.00

Chronic graft-versus-host disease grading*

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. Karnofsky or Lansky Clinical Performance scores, 60%, > 15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ systems as listed below.

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

* From Standard Practice Guidelines for “Chronic Graft-versus-Host Disease Classification at the time of presentation” developed by Long Term Follow-Up at the FHCRC

Laboratory testing and diagnostic indicators of chronic GVHD*

Eye	Schirmer's test with a mean value ≤ 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as FEV1 < 80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV1/FVC by > 12% within a period of less than 1 year. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans o the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/μl), eosinophilia, hypogammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

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

Appendix D - The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI)

Instructions: Circle applicable scores and provide actual value or cause of' co-morbidity.

Comorbidities	Definitions	HCT-CI weighted scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventriuclar arrhythmias	1	
Cardiac	Coronary artery disease‡, congestive heart failure, myocardial infarction, or EF≤50%	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis	1	
Diabetes*	Requiring treatment with insulin or oral hypoglycemics, but not diet alone	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular a ccident	1	
Psychiatric Disturbance	Depression anxiety requiring psychiatric consult or treatment	1	
Hepatic -mild*	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN,or AST/ALT >ULN-2.5XULN	1	
Obesity*	Patients with a body mass index > 35kg/ m^2	1	
Infection*	Requiring continuation of anti-microbial Treatment after day 0	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD Polymyalgia rheumatica	2	
Peptic ulcer*	Requiring treatment	2	
Moderate/severe renal*	serum creatinine>2mg/dl, on dialysis, or prior renal transplantation	2	
Moderate pulmonary*	DLCO and/or FEV, >65%-80% or Dispend on slight activity	2	
Prior solid tumor	*Treated at any time point in the patient's past history, excluding non-melanoma skin cancer*	3	
Heart valve disease*	Except mitral valve prolapse	3	
Severe pulmonary*	DLCO and/or FEV_1 <65% or dyspnea at rest requiring oxygen	3	
Moderate/severe Hepatic	Liver cirrhosis, Bilirubin>1.5XULN or AST/ALT>2.5XULN	3	
Please provide Karnofksy performance Score (KPS) =_____%		**Total Score =_____**	

Completed by (Print): _____ **Date:** _____

Signature: _____

*Comorbidity is currently active or patient requires medical treatment +

‡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 erythematosus; RA, rheumatoid arthritis; CTD, connective tissue disease;

DLCO, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransfcrase; ALT, alanine aminotransferas

Appendix E
Potential Adverse Events Associated or Expected with Hematopoietic Cell Transplantation

1. Graft versus host disease: GVHD is a major toxicity associated with the infusion of allogeneic donor stem cells. GVHD may be acute or chronic and may affect multiple organ systems, including the skin, liver, and GI tract.

2. Opportunistic infections, including viral and fungal infections, can result in severe pulmonary, neurologic, hepatic and other organ dysfunction, and possible death.

3. Gastrointestinal toxicity. Nausea and vomiting can be anticipated during the entire course of ablative therapy. Mucositis and diarrhea should be expected. Prednisone can cause GI bleeding.

4. Cardiac toxicity. Cardiotoxicity (congestive heart failure, pericardial effusion, EKG changes) is uncommonly associated with the chemotherapy agents and TBI used in the regimen and these sequelae may prove lethal.

5. Pulmonary toxicity. Diffuse interstitial pneumonitis of unknown etiology and diffuse alveolar hemorrhage occurs with some regularity after BMT and interstitial fibrosis occurs much more rarely. Both are well-described complications of intensive chemotherapy and TBI regimens and may prove lethal.

6. Hepatic toxicity. Veno-occlusive disease of the liver is a common toxicity of high-dose chemoradiotherapy and may result in death. Cyclosporine may cause elevation of ALT/AST.

7. Renal dysfunction. Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from cyclosporine and generally responds to dose reduction. Rarely, idiopathic or calcineurin inhibitor-associated hemolytic-uremic syndrome may occur and may be progressive and fatal. A syndrome of moderate renal insufficiency and hemolysis has been seen 5-7 months post HSCT after intensive multi-agent conditioning plus TBI.

8. Hemorrhagic cystitis, manifested either as gross or microscopic hematuria, is a common toxicity after high-dose chemoradiotherapy, but usually associated with regimens that include cyclophosphamide. Hemorrhagic cystitis may predispose to a long-term increased risk of bladder cancer.

9. Central nervous system toxicity. Radiation and chemotherapy can cause CNS toxicity, including seizures, depressed mental status, or leukoencephalopathy. Calcineurin inhibitors can cause seizures or other CNS toxicity.

10. Marrow aplasia. Severe neutropenia, thrombocytopenia, and anemia, is expected to occur for a period of 7 to 42 days following infusion of marrow. Transfusion of platelets and red blood cells is expected as supportive care. Transfusion of blood products may be associated with acquisition of HIV or a hepatitis virus. Neutropenia may increase the risk for acquiring serious infection. Thrombocytopenia may increase the risk of life-threatening hemorrhage. Hemorrhagic or infectious complications during the expected period of aplasia may result in death.

11. Miscellaneous. Alopecia and sterility are expected complications of the program as a whole. Cataract development is possible after TBI and/or steroids. Deficiencies of growth hormone, thyroid hormone, and sex hormones are possible after TBI. Calcineurin inhibitors can cause transient gingival hyperplasia, tremor, seizure, hypertension, headache, dysesthesia and hirsutism. Steroid therapy can also contribute to fluid retention, easy bruising, hypertension, aseptic necrosis of bone and increased susceptibility to infection. MMF can cause spontaneous abortions and birth defects. Hospitalization during conditioning and recovery period is expected to be 5-9 weeks in duration.

Appendix F - FHCRC IRB Policies

IRB Policy Web Page: http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

Adverse Events and Noncompliance are addressed in the following policies:

Policy 1.9: “Noncompliance”

Policy 1.11: “Reporting Obligations for Principal Investigators”

Policy 2.6: “Unanticipated Problems Involving Risks to Subjects or Others”

Appendix G Schedule of Study Evaluations

Data	1 year	2 year
Karnofsky/ECOG/Lansky	X	X
CBC w/differential	X	X
Disease restaging- BM aspirate (+/- biopsy as indicated)	X	
Chimerism – Bone Marrow	X	
Sorted Chimerism – Peripheral Blood	X	
IgA, IgG, IgM levels	X	X
Lymphocyte Panel (TCSNK)	X	X
GVHD Evaluation	X	X
Physical Exam	*If clinically indicated*	*If clinically indicated*
Viral screening	*If clinically indicated*	*If clinically indicated*
CMV Surveillance by PCR	*If clinically indicated*	*If clinically indicated*
PFT, MUGA/Echo, CT	*If clinically indicated*	*If clinically indicated*
Relapse	*Captured at occurrence*	
SAEs	*Captured at occurrence*	
Death	*Captured at occurrence*	

Effective as of this amendment, the schedule of activities that occurred prior to one year are shown in the prior protocol version. Every effort will be made to complete the 1 and 2 year evaluations as close to these dates as possible, taking into consideration the patient's circumstances.