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UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE
SEATTLE CHILDREN'S HOSPITAL

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Lead Principal Investigator:	Filippo Milano, MD, PhD Associate Professor, Fred Hutchinson Cancer Center, Clinical Research Division Associate Professor, Medical Oncology, University of Washington School of Medicine
Affiliate Investigator:	Ann Dahlberg, MD Associate Professor, Fred Hutchinson Cancer Center, Clinical Research Division Associate Professor, Department of Pediatrics, Hematology/Oncology, University of Washington School of Medicine Attending Physician, Seattle Children's Hospital
Study Biostatistician:	Wendy Leisenring, ScD Professor, Fred Hutchinson Cancer Center, Clinical Research Division Professor, Fred Hutchinson Cancer Center, Public Health Science Division
IND Sponsor:	Fred Hutchinson Cancer Center 1100 Fairview Ave N, M/S J3-329 Seattle, WA 98109-1024 (O): 206-667-4247 (F): 206-667-1370 ISIOC@fredhutch.org
Medical Monitor:	Monica Thakar, MD

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Authorized Sponsor Representative:
Name (typed or printed): Monica Thakar, MD
Institutional Title: Professor, ISIOC Medical Monitor
Institution: Fred Hutchinson Cancer Center

Signature: _____

Signature Page:

Investigator Statement:

I have carefully read Protocol RG9218003 entitled *Pilot Study: Infusion of off-the-shelf ex vivo expanded cryopreserved progenitor cells (dilanubicel) in the setting of single cord blood transplantation for patients with hematologic malignancies,* version date October 3, 2024.

I agree to carry out my responsibilities in accordance with the Protocol, applicable laws and regulations (including 21 CFR Part 312), Good Clinical Practice: Consolidated Guidance (ICH-E6[R2]), and applicable policies of Fred Hutchinson Cancer Center.

Principal Investigator: _____

Name (typed or printed): Filippo Milano, MD, PhD

Institution and Address: Fred Hutchinson Cancer Center
1100 Fairview Ave. N., Mailstop MD-B306
Seattle, WA 98109

Phone Number: 206.667.5925

Signature: _____ Date: _____

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1.0 INTRODUCTION TO THE PROTOCOL

1.1 Introduction

Cord blood (CB) is an effective and widely used source of stem cells for patients undergoing hematopoietic stem cell transplantation.[1-3] Importantly, CB offers some distinct advantages over conventional stem cell sources.[4,5] Because CB is collected at the time of birth and then cryopreserved until matched to a specific recipient, CB donors are readily available with no donor attrition. Furthermore, there is a greater tolerance for human leukocyte antigen (HLA) mismatching without an increase in graft versus host disease (GVHD),[6] thereby increasing the donor pool especially for minority or mixed ethnicity patients.[7] However, CB grafts contain approximately 1/10th the number of hematopoietic stem cells relative to a conventional bone marrow or peripheral blood stem cell graft, resulting in a known increased risk of delayed engraftment or graft failure and early post-transplant related morbidity and mortality. Thus, the low cell dose in a CB graft remains a significant barrier to the more widespread use of this stem cell source.[8]

One of the first strategies aimed at increasing the available cell dose for CB transplant (CBT) was the use of two CB donors for transplant, a "double" CBT.[9] This approach was found to be safe, resulted in a reduced incidence of primary graft failure and significantly advanced the CBT field making this a standard approach for adult patients. However, even with a double unit CB graft whereby the cell dose is doubled, there is still a significant delay in hematopoietic recovery. Infused CD34+ cell dose is felt to be the most important predictor of the kinetics of myeloid engraftment.[8] Furthermore, CBT patients are at a higher risk of early non-relapse mortality (NRM), particularly in those patients with a time to neutrophil engraftment of ≥26 days.[4] Moreover, an absolute neutrophil count (ANC) of >100 on any given day post stem cell transplant has been shown to be a critical threshold for a decreased risk of mortality before day 100 post-transplant.[10] Thus, the significant delay in myeloid recovery that is observed in CBT recipients remains a critical barrier to successful outcomes in the CBT setting.

Strategies aimed at shortening the time to neutrophil and platelet recovery after CBT are important for effective reduction of the high risk of non-relapse mortality that is experienced by CBT recipients and for improving overall survival (OS) and is an area of great unmet medical need.[11,12] We have successfully developed a novel and clinically feasible methodology utilizing an engineered Notch ligand for the ex vivo generation of increased numbers of CD34+ cells from CB units.[13] Results from Phase I and Phase II clinical trials have not only demonstrated the safety of this approach but more importantly demonstrated for the first time that rapid myeloid engraftment can be achieved faster than what observed after conventional CBT.[13] Now, in order to improve accessibility and feasibility of CBT, it is necessary to establish strategies that eliminate the need for a second cord blood unit without increasing risk to that patient. Prior to this trial, our off-the-shelf universal donor ex vivo expanded CB product, known as dilanubicel, was investigated in the setting of conventional CBT. More specifically, patients received a single or double CBT based on HLA typing and cell dose of the identified CB donor(s), with the vast majority of patients (11 out of 15) undergoing a double CBT (dCBT) as per standard practice. Dilanubicel would then be used in addition to the primary single or double CB graft source.

Having demonstrated that dilanubicel is safe to infuse as a universal donor with preliminary efficacy data demonstrating more rapid time to neutrophil and platelet recovery, decreased treatment-related mortality (TRM) and decreased severe acute GVHD in patients receiving dilanubicel compared to a concurrent control group, we now propose to explore the use of dilanubicel exclusively in the setting of single CBT with dilanubicel abrogating the need for a second CB unit/donor. With the use of this universal donor off-the-shelf stem cell product capable of improving time to neutrophil engraftment and decreasing transplant related complications, we aim to show the safety and feasibility of patients undergoing a single CBT where they would normally undergo a double CBT, with dilanubicel replacing the need for a second HLA-matched CB donor by providing a high CD34 cell dose (5-10 million CD34/kg) capable of rapid engraftment and bridging hematopoiesis. While we will continue to evaluate the safety of using dilanubicel in the CBT setting, the safety endpoint in this trial refers to sustained donor engraftment in the setting of a low dose single donor CBT + dilanubicel as the graft source.

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If successful, our approach could be a significant advancement in CBT enabling the use of single donor CBT, even with relatively low dosed units, thereby expanding access to more potential CB donors that are lower cell dose, but better HLA matched. Importantly, this would reduce the cost of the donor source. Furthermore, because dilanubicel is not a patient-specific cell therapy and is available as a pre-expanded and fully qualified cryopreserved cell therapy that can be infused on demand, there is no treatment delay for the patient while the product is being manufactured. This is a critical advantage to this approach versus a patient-specific process that requires real time manufacturing and delays the start of conditioning to account for product manufacturing, putting the patient at risk of disease relapse, infection or even product failure.

In summary, the use of dilanubicel with a single donor CB graft may address most of the current hurdles limiting the more widespread use of CBT by eliminating the need for a dCBT, improving the kinetics of engraftment (neutrophils and platelets), and reducing regimen related toxicities, TRM, time in the hospital, and transfusion requirements. This would also lead to improved health care economics of CBT compared to conventional CBT. Successful reduction of TRM and graft failure coupled with the unique clinical merits of CBT including its lower rate of relapse would further improve outcomes in CBT patients and have the potential to lead to more widespread adoption of CB as a stem cell source for transplantation.

1.2 Preclinical Data

Overview of Preclinical Studies

Throughout the preclinical development of this product, first for use as a patient-specific HLA-matched product that was infused fresh immediately after culture, followed by further development as a cryopreserved universal donor (non HLA-matched) product, functional studies using a xenograft immunodeficient mouse model have consistently demonstrated multi-lineage engraftment of our product supporting its use as a source of cells for bridging hematopoietic support.[14] It is important to note that the majority of the pre-clinical work done in the development of this product has utilized CD34+ cells pooled from multiple donors. The ability to develop this product as an immediately available, universal donor product required demonstration that the product could be cryopreserved after manufacturing for future use without compromising the functional repopulating ability of the product when thawed and infused. As discussed below in more detail, preclinical studies have demonstrated that the final ex vivo expanded cellular therapy can be cryopreserved and infused after thaw without loss of repopulating ability as evidenced by multilineage engraftment in an immunodeficient xenograft mouse model. Additional preclinical work has been performed using a murine model with murine hematopoietic stem and progenitor cells (HSPC) that have been similarly expanded in the presence of Notch ligand and then cryopreserved. This work demonstrated the ability of cryopreserved, Notch-expanded murine hematopoietic stem and progenitor cells to overcome histo-incompatibility providing short-term engraftment and prolonging survival when transplanted into major H-2 mismatched recipients after lethal dose radiation.[15]

In vivo repopulating ability is retained following cryopreservation of the expanded cell product

Several validation experiments were performed 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, 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 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

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human engraftment of 8% at 2 weeks post infusion and 7% at 4 weeks post infusion. Lastly, we compared various thawing methodologies (thaw and wash, thaw and dilute with dextran/HSA and thaw and directly infuse) and did not observe a significant difference in the three methods evaluated (**Figure 2**).

Figure 1: In vivo repopulating ability of cultured, cryopreserved cells.

1a **1b**

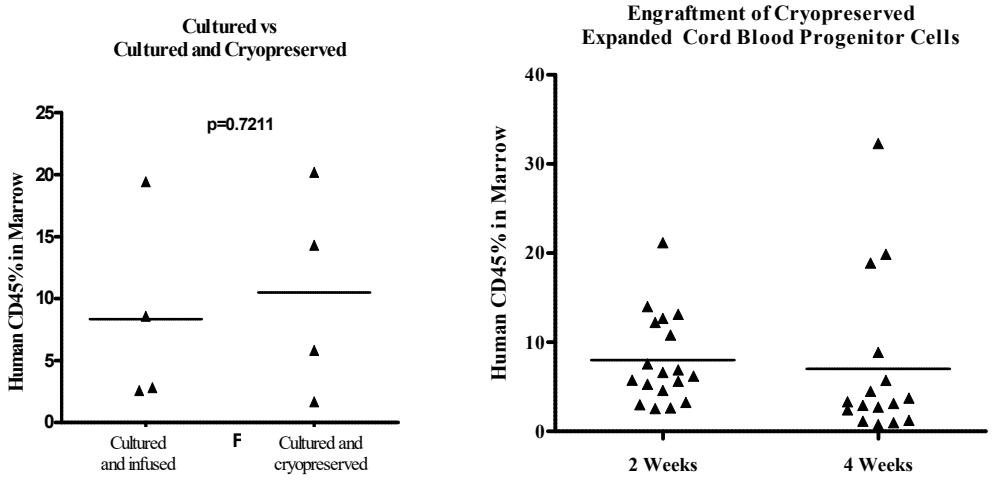


Figure 2: Cryopreservation of ex vivo expanded cord blood progenitors does not impair in vivo repopulating ability

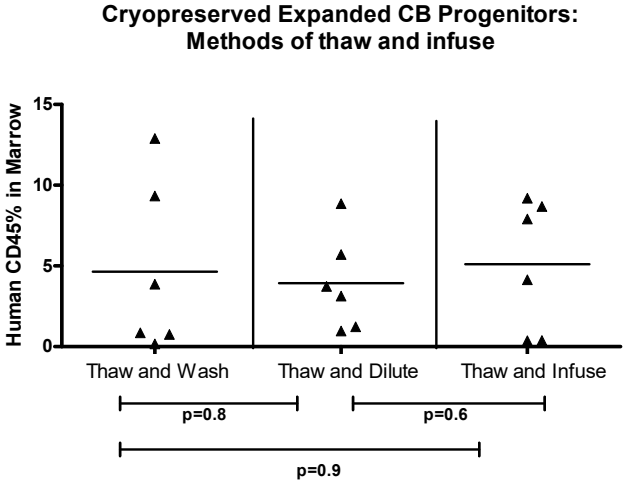


Figure 2: CD34^{+} cord blood progenitor cells were enriched and placed into culture with Delta1$^{ext\text{-}IgG}$ as described. Overall human engraftment as measured by human CD45 in the marrow of recipient mice is shown on the y axis. The solid lines represent the mean level of human engraftment. (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.

Notch-Expanded Murine Hematopoietic Stem and Progenitor Cells Improve Survival after Exposure to Lethal Radiation in Mismatched Recipients

Using a murine model of hematopoietic syndrome of acute radiation syndrome (h-ARS), we demonstrated that infusion of ex vivo expanded murine hematopoietic stem and progenitor cells (HSPCs) into major histocompatibility complex mismatched recipient mice exposed to a lethal dose of ionizing radiation led to rapid myeloid recovery and improved survival. Relevant to the current study, survival benefit was significant in a dose-dependent manner even when infusion of the expanded cell therapy was delayed 3 days after lethal IR exposure. Most surviving mice (80%) demonstrated long-term in vivo persistence of donor T cells at low levels, and none had evidence of graft versus host disease. These findings provide evidence that ex vivo expanded mismatched HSPCs can provide rapid, high-level hematopoietic reconstitution and mitigate IR-induced mortality in a murine h-ARS model.[15]

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1.3 Clinical Data

Proof of Principal: Preliminary Results of a Phase I CBT Trial Using Ex Vivo Expanded Cord Blood Progenitors (Fred Hutch 2044)

The first clinical trial using Notch-mediated e*x vivo* expanded CB HSPC was initiated in 2006 in the myeloablative double CBT (dCBT) setting, in which one of the two selected CB units underwent CD34 selection and *ex vivo* expansion prior to infusion. The T cells (negative fraction) were *not* reinfused. Although this trial was a first in human safety trial, the use of Notch ligand for clinically relevant *ex vivo* generation of increased numbers of HSPC was demonstrated. Furthermore, this trial was the first trial conducted that showed that infusion of *ex vivo* expanded CB HSPC, without co- infusion of T cells from the same donor, could result in a significant decrease in the time to hematopoietic recovery of both neutrophils and platelets. In fact, a significant reduction in the median time to an absolute neutrophil count (ANC) of 500/µl to just 11 days was observed compared to 25 days in patients not receiving expanded cells, and reduction in time to platelet recovery of 35 days versus 46 days (updated data).[13]

However, this approach of real time, on demand, current Good Manufacturing Practices (cGMP)-compliant expansions starting from previously cryopreserved units that are at least 4/6 HLA-matched to the recipient is not clinically feasible outside of highly specialized transplant centers. This approach is further complicated by manufacturing on a tight timeline dictated by the patient's treatment, not allowing for efficient usage of resources, facilities and personnel. There is an inherent risk to the patient that comes with this approach as treatment must be delayed to account for acquisition of the patient specific CB unit for expansion, manufacturing the expanded cell product, and then shipment of that product to the transplant center. There is also a risk of manufacturing failure or sterility contamination, leaving the patient with no alternative but a second, non-expanded unit or further delay of transplant until a new 4/6 matched CB unit can be identified and expanded.

In contrast, development of an "off-the-shelf" pre-expanded cellular therapy would obviate the requirement for real time production, and with no requirement for HLA-matching, would permit maintenance of a minimal inventory of expanded products that are immediately available for patients regardless of ethnicity. Furthermore, there is no treatment-delay associated risk to the patient with this approach as the expanded cell product is manufactured ahead of time and is available as a fully qualified (fully released), pre-expanded and cryopreserved product available on demand. Because our expanded cell product is derived from a purified population of CD34+ HSPC and does not contain donor T cells, and in order to overcome the limitations of patient specific product manufacturing, we went on to develop this product as a universal donor product. This cryopreserved universal donor product is referred to as dilanubicel. Prior to March 2017, the product was referred to as Delta cultured cryopreserved-umbilical cord blood (DCC-UCB). In the following discussions of clinical studies performed prior to March 2017 the product will be referenced as "DCC-UCB".

Results from a Pilot Study using DCC-UCB in the setting of myeloablative CBT (Fred Hutch 2378)

Fred Hutch Protocol 2378 aimed to evaluate whether a non-HLA matched, "off-the-shelf" version of the product could also support rapid myeloid recovery. In this pilot study, patients received either a single or double ablative CBT plus infusion of DCC-UCB. Fifteen patients were enrolled in this single center pilot study (see **Table 1** for patient/graft characteristics).[16]

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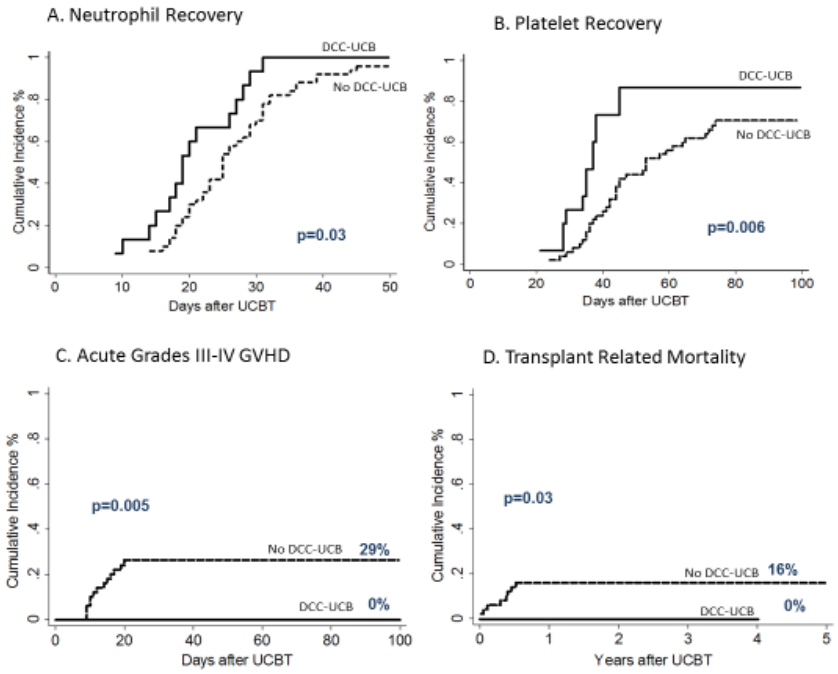
Table 1. Unit and patient characteristics in recipients of DCC-UCB and Fred Hutch historical controls

Patient Characteristics	DCC-UCB (n=15)	Controls (n=50)	p-value
Gender, No (%)			
Female	7 (46)	25 (50)	1
Age in years, median (range)	21 (5 to 45)	21 (0.6 to 43)	0.58
Weight in kilograms, median (range)	59 (23 to 89)	67 (8 to 109)	0.31
Diagnosis, No (%)			
ALL	8 (53)	16 (32)	
AML	6 (40)	24 (48)	0.61
MDS/CML	1 (7)	6 (12)	
Other	0	4 (8)	
CMV seropositive, no. (%)	11 (73)	31 (50)	0.47
Race, no. (%)			
Caucasian	6 (40)	21 (42)	0.22
Other	9 (60)	29 (58)	
Disease Risk			
Low	-	6 (12)	
Intermediate	11 (74)	30 (60)	0.37
High	3 (20)	13 (26)	
Very high	1 (6)	1 (2)	
MRD	6 (40)	19 (38)	0.88
Follow-up in years, median (range)	3.8 (2.4 to 4.3)	6 (1.7 to 8.8)	
Unit Characteristics			
Number of unmanipulated donors, No(%)			
1	4 (27)	9 (18)	
2	11 (73)	41 (82)	0.46
HLA match unmanipulated donors, No (%)*			
4/6	9 (60)	29 (58)	0.60
5/6	6 (40)	16 (32)	
6/6	.	5 (10)	
Infused Cell Doses (pre-freeze)			
Total Unmanipulated TNC/kg x 10^7	6.1 (4.3 to 17.1)	4.9 (3.4 to 16.6)	0.03
Total Unmanipulated CD34/kg x 10^6	0.26 (0.08 to 0.98)	0.19 (0.04 to 0.98)	0.07
Expanded Product TNC/kg x 10^7	5.8 (2.2 to 10.9)	.	
Expanded Product CD34/kg x 10^6	5.2 (3.1 to 11.6)	.	

* HLA matching reflects the lowest HLA-match of the unmanipulated unit

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Figure 3: Infusion of DCC-UCB results in faster hematopoietic recovery (A, B) and reduced severe acute GVHD (C) and TRM (D).



The results of this pilot study demonstrated that infusion of DCC-UCB to augment a CB graft in the myeloablative setting was safe and led to more rapid neutrophil and platelet recovery compared to our concurrent control group. Importantly, no TRM was observed among the 15 patients on this study, with only 2 deaths due to relapsed disease, leading to an excellent OS of 86% at a median follow-up of 3.2 years. Quite interesting was the observation of no grade 3-4 acute GVHD, even with infusion of a non-HLA matched expanded cell product that theoretically may induce increased alloreactivity of the unmanipulated CB unit(s). In this pilot study, it appeared that infusion of DCC-UCB was associated with less severe acute GVHD. Based on these promising results, a prospective multicenter randomized trial (Fred Hutch Protocol 2603) utilizing this product is underway.

Chemotherapy-induced neutropenia (Fred Hutch 2335)

In addition to these studies in the CBT setting, a Phase I trial was conducted at Fred Hutch investigating the safety and preliminary efficacy of infusing DCC-UCB following intensive chemotherapy for Acute Myeloid Leukemia (AML). This trial enrolled 29 adult AML subjects (19 newly diagnosed and 10 relapsed/refractory), testing the safety of infusing dilanubicel after induction and consolidation chemotherapy with clofarabine, cytarabine and G-CSF priming (GCLAC). No unexpected toxicity or transfusion-related graft-versus-host-disease was observed. There was no evidence of in vivo persistence of the DCC-UCB product in any subject beyond 14 days after infusion. Additionally, there was no evidence of DCC-UCB induced alloimmunization.

Results were compared to 106 subjects given GCLAC alone during the same time period. Documented infections were observed in 34% of subjects receiving dilanubicel versus 57% receiving GCLAC alone (p=0.03) after chemotherapy cycle one and 8% versus 52% (p=0.004) in subsequent courses with reduction in risk of documented infection in the group receiving dilanubicel (in particular gram-negative rod associated bacteremia). Despite the decreased infection rate, fever of unknown origin (FUO) was more frequent among subjects receiving DCC-UCB and increased with higher dilanubicel cell dose. However, median time of FUO was significantly shorter in the DCC-UCB group.[17]

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Additional Results in Pediatric Population (Fred Hutch 2584)

A Phase I trial to investigate the safety of infusing DCC-UCB in pediatric and young adult patients following chemotherapy with fludarabine, cytarabine, and G-CSF (FLAG) to treat AML or acute leukemia of ambiguous lineage was also held at Fred Hutch. The study planned to enroll 15 patients but closed after 7 due to a slow rate of accrual and change in institutional prioritization for this patient population. All patients enrolled received the protocol-specified FLAG chemotherapy and DCC-UCB infusion at a median cell dose of 9.58 x 10^6 CD34/kg and 7.66 x 10^7 TNC/kg. Infusion in this population was found to be generally well tolerated without any infusion toxicities identified among the 7 patients enrolled. No correlation of DCC-UCB dose on time to neutrophil or platelet recovery was identified in this study because of the limited population.

Analysis of chimerism results showed that no patient experienced persistence of DCC-UCB product past one week post infusion. No stopping rule events occurred, and no patient reported an SAE or fatal adverse event, though there were 5 deaths ranging from Day 200 to 1159 post-infusion all for reasons related to progressive disease or relapse.

1.4 Study Agent (dilanubicel)

Dilanubicel is a cryopreserved cell therapy composed of ex vivo expanded CD34+ hematopoietic stem and progenitor cells originally isolated from umbilical cord blood units screened for donor eligibility per 21 CFR 1271 and then cultured in the presence of the Notch ligand, Delta1. Dilanubicel contains no T cells and is used as an off-the-shelf, universal donor product with no requirement for HLA matching. This cryopreserved universal donor product is referred to as dilanubicel. Prior to March 2017, the product was referred to as Delta cultured cryopreserved-umbilical cord blood (DCC-UCB). The intended use of dilanubicel is to reduce the morbidity and mortality associated with prolonged cytopenia after cord blood transplantation for treatment of hematologic malignancies. Dilanubicel products are fully tested and released for clinical use prior to being added to available inventory. Refer to dilanubicel Investigator Brochure for product details.

1.5 Dose Rationale

Dilanubicel is to be infused as a single dose infusion of 800 million total CD34+ cells administered after any toxicities related to infusion of the primary single donor CB graft are resolved. For an average 75 kg patient, these cell numbers would correspond to 10.7 x 10^6/kg. The recommended dose comes from our prior studies in CBT using this cell therapy as a patient specific product and as a universal donor product, where doses ranged from 0.6 – 49 million CD34/kg. Please see **Table 2** below for more details.

Table 2. Prior CBT studies and CD34+ dose ranges

Study	CD34+ cell dose/kg
Protocol 2044 (n=22)	Mean: 7.7 x 10^6 Range: 0.6 to 49.5 x 10^6
Protocol 2378 (n=15, 15 doses)	Mean: 5.2 x 10^6 Range: 3.1 to 11.6 x 10^6

As discussed above, it is important to note that dilanubicel contains no mature T cells, and therefore should be able to be infused in a recipient as a non HLA-matched product with the intent of providing only an initial wave of rapid short-term engraftment. As in the previous trial, we do not anticipate or intend for the expanded product (dilanubicel) to be the long-term source of sustained donor hematopoiesis due to likely eventual (or immediate) rejection mediated by the non-manipulated unit. No safety issues infusing these products have been identified in the previously conducted pilot study (Fred Hutch 2378) or the ongoing randomized Phase II trial (Fred Hutch 2603).

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1.6 Other Agents

Preparative regimens, including regimen rationale

This clinical trial will allow a choice of one of two myeloablative preparative regimens (see Section 9.2). The regimens were selected based on prior experience and use of these regimens in the "standard of care" myeloablative CBT setting. The protocol allowed conditioning regimens are:

1. Regimen A: High dose consisting of 1320 cGy TBI + Fludarabine + Cyclophosphamide
2. Regimen B: Intermediate dose consisting of 400 cGy TBI +Fludarabine + Cyclophosphamide + Thiotepa

Regimen A is a commonly used myeloablative regimen used for patients 45 years and younger undergoing CBT for high-risk hematologic malignancies. It has been in clinical use for more than 15 years nationally and internationally is a standard of care regimen. However, this regimen is also associated with extensive toxicities, with known risk factors for end organ toxicity, restricting the use of high dose TBI to younger (< 46 years) patients without significant pre-existing co-morbidities. 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. Thus, there was a significant need to develop a well-tolerated intermediate intensity myeloablative regimen to address the significant limitations of the high dose TBI and nonmyeloablative regimens.

Regimen B was developed by Dr. Juliet Barker at Memorial Sloan Kettering Cancer Center (MSKCC) as an alternative 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). Dr. Barker has extensive experience with this regimen, published for the first time in 2013 which presented results for 30 patients with acute leukemia and myelodysplasia (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) which is nearly identical to that observed using the high dose TBI based regimen. Furthermore, the reported rate of TRM at day 180 was 20%, similar to the conventional high dose TBI regimen.[18] These results demonstrate that this is a promising alternative myeloablative regimen to high dose TBI based conditioning and expands the benefits of a myeloablative conditioning regimen to a wider cohort, in particular to patients 50 years and older who are otherwise reasonably fit, without increasing risk.

The regimen selection for individual subjects is flexible to allow for attending physician preference. There are some absolute criteria, for example, that subjects between the ages of 46 and 65 would only be eligible for the intermediate intensity regimen. However, outside of this, the decision of which conditioning regimen would be based on the underlying disease, presence of minimal residual disease age, and co-morbidities. Furthermore, there is also a move away from high dose TBI by some physicians in younger subjects 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 subject specific factors that lead to the specific regimen choice by the physician. In general, it is anticipated that younger subjects, especially those with minimal residual disease, will accrue to the high dose TBI regimen, and the intermediate intensity regimen will accrue those subjects between 46 and 65 years of age and subjects for whom the attending physician feels this is the more appropriate regimen.

Use of a single donor non-manipulated cord blood graft

Today, most adult patients and larger children routinely receive a cord blood graft consisting of two unrelated donor cord blood units to meet cell dose thresholds and reliably achieve sustained donor engraftment. Despite the use of two individual cord blood grafts, hematopoietic recovery remains significantly delayed and single donor dominance is observed in nearly all patients as a result of immune mediated rejection of one unit by the other with only one of two units engrafting as the source of long term hematopoiesis.[19] Furthermore, a major limitation of the use of CB as a stem cell source is the cost associated with acquiring two cord blood units.

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In this study, we are evaluating the safety of using a single unmanipulated CB unit as the primary graft source in all adult patients when infused with dilanubicel intended to function as the source of rapid bridging hematopoiesis until engraftment from the primary cord blood unit occurs. The single donor cord blood unit will be required to have a minimum total nucleated cell (TNC) dose of 2.5 x 10^7 TNC/kg, CD34 cell dose of 1.7 x 10^5 CD34/kg, and as per standard of care, be HLA matched at a minimum of 4/6 HLA based on intermediate resolution at HLA-A and B and high resolution at HLA-DRB1. The choice for the minimum TNC dose is based on previous studies showing that CBT recipients receiving < 2.5 x 10^7TNC/kg had worse clinical outcomes when compared with patients receiving a higher TNC dose.[20] Similarly, Wagner et al. have provided more insight on treatment outcomes based on CD34+ dose especially with respect to the kinetics of engraftment. Patients undergoing myeloablative CBT and receiving an infused CD34 cell dose of <1.7×10^5 cells/kg had inferior outcomes when compared to patients receiving a higher CD34 cell dose.[8]

Current standard of care donor selection for CBT is based on both cell dose and HLA, with higher cell doses required for CB units that are more mismatched. Thus, as it stands, it is currently very difficult for patients to find a single CB unit that meets the criteria for use as a single donor. Based on our knowledge of more than 60 patients who have received dilanubicel in the myeloablative CBT setting and the ability of this product to provide rapid but short-term engraftment, we now propose to evaluate the use of this product as a bridging graft source for patients undergoing single donor myeloablative CBT. It should be noted however that patients will not be eligible unless they can identify a suitably matched single CB unit that is at least 2.5 x 10^7 TNC/kg. This is in contrast to current standard of care in which patients without a suitable single CB donor can go on to receive a double unit CBT with two units of TNC cell dose at least 1.5 x 10^7 TNC/kg. Future trials will determine the minimum cell dose required for the single donor CB graft.

GVHD prophylaxis and supportive care

Supportive care [(including transfusions, infection prophylaxis (bacterial, fungal, viral), and other therapy)] will be in accordance with institutional guidelines. GVHD prophylaxis will consist of cyclosporine (CSA) and mycophenolate mofetil (MMF) per institutional guidelines. See Section 9.3 for details.

1.7 Risks/Benefits

1.7.1 Potential Risks

Please refer to the Investigator's Brochure for details.

As an off-the-shelf, non-HLA matched cellular therapy, theoretical concerns regarding the infusion of expanded cells exist. These include the possibility of infusion toxicity, increased rates of acute or chronic GVHD, alloimmunization, and immediate rejection of the expanded product. Greater than 100 infusions of dilanubicel have been performed in the setting of cord blood transplant and chemotherapy induced neutropenia with no unanticipated adverse events. However, we will closely monitor the safety of this universal donor product as outlined in this protocol.

Infusion toxicity

Our first trial used HLA-matched products with cell doses that went as high as 13.9x10^7 TNC/kg and 49 x 10^6 CD34/kg with no noted infusion reactions. Subsequent CBT studies have used dilanubicel as an off-the-shelf universal donor product that is cryopreserved. The cell dose range of dilanubicel for Protocol 2378 was 2.2x10^7 TNC/kg to 10.9x10^7 TNC/kg and 3.1x10^6 CD34/kg to 11.6x10^6 CD34/kg. The ongoing randomized protocol (Protocol 2603) has utilized similar cell dose ranges. However, the possibility of infusional toxicity exists and as such, monitoring requirements are included in this protocol from infusion through 24 hours.

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Increased Rates of Acute or Chronic GVHD

Subjects enrolled in this protocol will have been treated with a standard of care myeloablative preparative regimen and infusion of a cord blood donor that has been selected for the recipient on the basis of HLA-matching and cell dose. All patients will also receive dilanubicel which is an off-the-shelf universal donor cell therapy product selected without respect to HLA. Increased risk of GVHD associated with dilanubicel directly is very unlikely as the expanded product is derived from a population of purified CD34+ cells and does not contain any T cells. Furthermore, no mature T cells are generated in culture. However, it is possible that infusion of dilanubicel in addition to the intended cord blood donor may increase the risk of developing acute or chronic GVHD over baseline rates by enhancing the alloreactivity of T cells from the unmanipulated CB donor. GVHD outcomes will be closely monitored in this study.

Alloimmunization

Although there was no evidence of alloimmunization that can be directly attributed to dilanubicel, alloimmunization is a possibility; therefore, it will be assessed in this study.

Immediate rejection of dilanubicel

Cord blood donor selection for the unmanipulated unit will follow the umbilical cord blood graft selection algorithm in Section 6.0 that is based on both HLA-typing and cell dose. The dilanubicel product is used to augment this conventional CB graft with the goal of providing rapid hematopoietic recovery, in particular myeloid, and platelet recovery, until the unmanipulated donor engrafts. It is possible that there could be near immediate immune-mediated rejection of the expanded cell product. However, it is possible based on previous studies that the product may enhance the engraftment of the single unit graft and have other beneficial effects independent of engraftment. Also, based on stringent criteria for selection of a quality product for the unmanipulated unit engraftment is expected as would normally occur after adequately dosed single unit grafts.

Graft failure

Graft failure is a possibility. All patients undergoing allogeneic stem cell transplant with any donor source of stem cells are at risk for graft failure. In this protocol specifically, primary graft failure will be used as a measure of safety in patients undergoing single donor CBT with infusion of dilanubicel as the source of bridging hematopoiesis. Primary graft failure for patients undergoing myeloablative CBT ranges between 10-20%. The risk of graft failure may be higher in this study with the use of a single cord blood unit. See Section 16 for stopping rules based on primary graft failure and GVHD.

1.7.2 Potential Benefits

As discussed above, the use of CB as a source of hematopoietic stem cells for transplantation offers many advantages, especially for those subjects of minority or mixed ethnicity background (and 30-40% of Caucasians) who cannot identify a suitably matched adult volunteer donor. Our focus is on overcoming the delay in engraftment experienced by subjects undergoing a cord blood transplant, with the goal of improving subject outcomes.

Having demonstrated safety and preliminary efficacy in terms of hematopoietic recovery, reduction of transplant related mortality and severe acute GVHD, and improving DFS when infusing dilanubicel in the setting of conventional single or double myeloablative CBT, we are now focused on evaluating the safety of single unit CBT in adult patients when dilanubicel is also infused. The intent of dilanubicel is to provide a source of rapid bridging (short-term) hematopoiesis until long term engraftment occurs from the single donor CB graft. This approach aims to overcome the delay in hematopoietic recovery and consequently reduce the associated risks of morbidity and mortality. Additional potential benefit may be observed in lower rates of acute GVHD by using only a single donor for CBT (double unit CBT has been associated with increased rates of acute GVHD)[6]. Finally, if

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this approach is shown to be safe and effective, this could abrogate the need for a second unit. Future studies would then evaluate the minimal effective cell dose for the single donor CB graft when infused with dilanubicel. This could have a dramatic impact on clinical practice and open up the possibility of utilizing banked cord blood units that have previously been considered too small for use in transplantation, as well as decreasing the cost of CBT by reducing the cost of the CB graft.

2.0 OVERVIEW OF CLINICAL TRIAL

2.1 Study Objectives

2.1.1 Primary Objectives

The primary objective of this study is to show that single unit cord blood transplantation augmented with a single dose of dilanubicel is not associated with an excess of graft failure.

2.1.2 Secondary Objectives

1. Examine the safety and toxicity of infusing dilanubicel
2. Evaluate neutrophil and platelet engraftment
3. Determine incidence of acute GVHD at day 100 and chronic GVHD at 1 and 2 years
4. Determine non-relapse mortality at day 100 and 180

2.1.3 Exploratory Objectives

1. Evaluate in vivo persistence of dilanubicel
2. Determine duration of initial hospitalization from day 0 post-transplant
3. Determine overall survival at 1 year
4. Immune reconstitution: assess the kinetics of immune system recovery as measured by T and B cell subsets as possible with available clinical data collected as part of standard of care

2.2 Study Endpoints

2.2.1 Primary Endpoint

Primary graft failure/rejection as defined by no neutrophil recovery by day 45 (regardless of donor chimerism) or autologous recovery (neutrophil recovery but < 10% donor chimerism in blood and BM) by day 45.

2.2.2 Secondary Endpoints

1. CTCAE Version 5.0 Grade ≥ 3 infusion toxicity, defined as grade 3+ events within the first 24 hours after infusion
2. Neutrophil recovery will be the 1st day of 2 consecutive days of absolute neutrophil count at or above 500 after the 1st post-CBT nadir and platelet engraftment will be defined as the first day of a platelet count > 20,000/µl without subsequent transfusions for 7 days
3. Severe (grades III-IV) acute graft-versus-host disease (GVHD) and Chronic GVHD defined by the 2014 NIH criteria
4. Non-relapse mortality, defined as death without a prior relapse

2.2.3 Exploratory Endpoints

1. In vivo persistence of dilanubicel
2. Duration of initial hospitalization from day 0 post-transplant
3. Overall survival at 1 year

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4. Immune reconstitution: assess the kinetics of immune system recovery as measured by T and B cell subsets

2.3 Estimated Accrual

Thirty-five participants will receive the investigational product under this protocol.

3.0 SAFETY CONSIDERATIONS

See Section 16.0, Statistical Considerations for stopping rules.

4.0 BRIEF DESCRIPTION OF MANUFACTURING PROCESS FOR DILANUBICEL

Dilanubicel is a universal donor "off-the-shelf" human stem/progenitor cell product that is cryopreserved and ready for immediate use. Dilanubicel is manufactured from CD34+ hematopoietic stem and progenitor cells (HSPCs) isolated from cord blood units that have been screened for donor eligibility per 21 CFR 1271. This manufacturing process is conducted under BB-IND 14184.

In brief, dilanubicel is derived from human CD34+ cells that are isolated from donor eligible cord blood units and placed into culture for 16 days in the presence of recombinant human cytokines and immobilized Notch ligand. Following culture, cells are harvested, concentrated, removed from culture media and then resuspended in a cryoprotectant solution containing $\leq$ 10% Dimethylsulfoxide (DMSO). Cells prepared for cryopreservation are transferred to sterile cryopreservation bags. The dilanubicel product is frozen in a controlled-rate freezer and transferred to storage in a vapor-phase liquid nitrogen (LN_2) freezer. Dilanubicel products are fully tested and released for clinical use prior to being added to available inventory.

5.0 SUBJECT ELIGIBILITY

5.1 Inclusion Criteria

Patients 10 to 65 years old with a hematologic malignancy in need of hematopoietic cell transplant who are > 30 kg and without a suitable related donor.

5.1.1 Disease Criteria

A. Patient must have hematologic malignancy that meets institutional eligibility requirements for cord blood transplant.
B. Malignancies included are:
    i. Acute Leukemia, including Acute Myeloid Leukemia (AML), Biphenotypic Acute Leukemia or Mixed-Lineage Leukemia, and Acute Lymphoblastic Leukemia (ALL). All patients must be in CR as defined by < 5% blasts by morphology/flow cytometry in a representative bone marrow sample with adequate cellularity to assess remission status.
    ii. 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 morphology in a representative bone marrow aspirate.
    iii. Chronic Myeloid 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.

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5.1.2 Age criteria

The age criteria will be dependent on the conditioning regimen selected.

A. High dose TBI regimen: 10 to ≤ 45 years
B. Intermediate Intensity regimen: 10 to ≤ 65 years

Conditioning regimen selection should be based on the underlying disease and remission status including presence of minimal residual disease, age, co-morbidities, attending physician, and site preference.

5.1.3 Organ Function and Performance Status Criteria

A. Performance status score:
    i. Patients 10 to ≤ 45 years: Lansky (< 16 years old) or Karnofsky (≥ 16 years old) ≥ 70 or ECOG 0-1 (see **Appendix A**)
    ii. Patients > 45 to ≤ 65 years: Karnofsky ≥ 70 or ECOG 0-1 and non-age adjusted comorbidity index ≤ 5 (see **Appendix D**)
B. Renal Function
    i. Adults: Calculated creatinine clearance must be > 60 mL and serum creatinine ≤ 2 mg/dL
    ii. Children (<18 years old): Calculated creatinine clearance must be > 60 mL/min
C. Hepatic Function
    i. Total serum bilirubin must be < 3mg/dL unless the elevation is thought to be due to Gilbert's disease or hemolysis
    ii. Transaminases must be < 3x the upper limit of normal per reference values of treating institution
D. Pulmonary function
    i. DLCO corrected ≥ 60% normal (may not be on supplemental oxygen)
    ii. For pediatric patients unable to perform pulmonary function tests, O2 saturation > 92% on room air
E. Cardiac function
    i. Left ventricular ejection fraction ≥ 50% OR
    ii. Shortening fraction > 26%
F. Ability of participant or legally authorized representative to understand and the willingness to sign a written informed consent form

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 unless cleared by ID consult
C. History of HIV infection
D. Pregnant or breastfeeding
E. Prior allogeneic transplant
F. CNS leukemic involvement not clearing with intrathecal chemotherapy. Diagnostic lumbar puncture is to be performed as per Section 9.6
G. < 30 kg

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6.0 CORD BLOOD DONOR 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 CB donor based on, but not exclusively determined by, cell dose (TNC/kg and CD34/kg), HLA matching, disease status, and indication for transplant.

A. HLA matching:
    1. Minimum requirement: The CB unit must be matched at a minimum at 4/6 HLA-A and B antigens and DRB1 allele with the recipient. Therefore, 0-2 mismatches at the A, B, or DRB1 loci based on intermediate resolution at HLA-A, B and high-resolution allele level typing at HLA-DRB1 are allowed.
    2. Institutional guidelines for HLA-match may be followed as long as the minimum criteria for HLA-matching as above are met.

B. Cell Dose Requirements:
    1. The CB unit selected for transplant must have a MINIMUM of 2.5 x 10^7TNC/kg.
    2. The minimum recommended CD34/kg cell dose is 1.7 x 10^5 CD34/kg.
    3. A backup unit must be identified and reserved prior to the start of the treatment plan for possible infusion in the unlikely event of poor post-thaw viability of the primary CB unit. A suitable back up unit will be considered, as follows:
        i. 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 (Fred Hutch Protocol 2010- Appendix V).
        ii. Must contain a MINIMUM of 1.5 x 10^7 TNC/kg to ensure the same requirement we use for a standard double CBT per CB selection guideline (Fred Hutch Protocol 2010- Appendix V).

6.2 Dilanubicel – Investigational Agent

Dilanubicel is manufactured according to BB-IND 14184 and as described in the protocol previously. Each dose contains 800 million total viable CD34+ cells in approximately 20 mL at the time of cryopreservation. All patients will receive one dose of dilanubicel, infused after the primary single donor graft.

All products are manufactured and released for clinical use. Refer to Protocol 9910 Handling and Administration Guidelines for instructions.

7.0 INFORMED CONSENT OF SUBJECTS

Before initiating research-specific procedures that would not otherwise be done for a subject, the investigator must discuss the protocol thoroughly with the subject or his/her legally authorized representative, including all known risks to the subject. 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 his/her legally authorized representative. Informed consent will be obtained using consent forms approved by the Institutional Review Board (IRB). The consent form must be signed and dated by the subject or his/her legally authorized representative. The case history for each subject will document that informed consent was obtained before the

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subject's study participation, detailing what was covered. A copy of the consent form must be provided to the subject. Signed consent forms must remain in each subject's chart and must be available for verification by monitors or regulatory agencies at any time.

8.0 SUBJECT REGISTRATION

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

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. Subjects will be registered by the Fred Hutch/University of Washington (UW) Study Coordinator and entered into the Clinical Trial Management System (CTMS). Complete, signed study consent and HIPAA authorization forms are required for registration.

9.0 TREATMENT PLAN

9.1 Conditioning Regimens

There are two conditioning regimens allowed per protocol:

1. Regimen A: High dose consisting of 1320 cGy TBI + Fludarabine + Cyclophosphamide.
2. Regimen B: Intermediate dose consisting of 400 cGy TBI + Fludarabine + Cyclophosphamide + Thiotepa.

REGIMEN A: High Dose TBI regimen (must be 10 to ≤ 45 years**)**

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 9.2A)
-2	TBI 165 cGy twice daily	
-1	TBI 165 cGy twice daily	
0	**CBT**: Unmanipulated unit(s) (umbilical cord blood), followed by infusion of dilanubicel at a minimum of 4 hours after **completion*** of unmanipulated unit	Begin MMF (as per Section 9.2B)
+1		Begin G-CSF (as per Section 9.3A

* See 9.5.B

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A. Fludarabine:
   1. 25 mg/m^2/day IV over 30 minutes x 3 days (days -8 to -6); total dose 75 mg/m^2.
   2. Preparation, administration and monitoring will be according to institutional guidelines. For subjects > 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 120 mg/kg.
   2. Preparation, administration and monitoring will be according to institutional guidelines. If subject'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 Fred Hutch 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: Intermediate Intensity regimen (10 to ≤ 65 years**)**

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 -4), 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 2-4 hours	
-4	Fludarabine 30 mg/m^2 IV over 30-60 minutes Thiotepa 5 mg/kg IV over 2-4 hours	
-3	Fludarabine 30 mg/m^2 IV over 30-60 minutes	Begin CSA (as per Section 9.2A)
-2	Fludarabine 30 mg/m^2 IV over 30 minutes TBI 200 cGy once daily	
-1	TBI 200 cGy once daily	
0	**CBT**: Unmanipulated unit(s) (umbilical cord blood), followed by infusion of dilanubicel at a minimum of 4 hours after **completion*** of last unmanipulated unit	Begin MMF (as per Section 9.2B)
+1		Begin G-CSF (as per Section 9.3A)

*See 9.5B.

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A. Fludarabine:
   1. 30 mg/m^2/day IV over 30 minutes x 5 days (days -6 to -2); total dose 150 mg/m^2.
   2. Preparation, administration and monitoring will be according to institutional guidelines. For subjects > 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, dose will be calculated using adjusted weight.

C. Cyclophosphamide:
   1. 50 mg/kg IV x1 day (day -6)
   2. Preparation, administration and monitoring will be according to institutional guidelines. If subject'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 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).

9.2 Immunosuppressive Therapies

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

A. **Cyclosporine (CSA)**

1. Subjects will receive cyclosporine (CSA) therapy beginning on day -3, maintaining a level of >200 ng/mL. The initial dose will be 2.5 mg/kg IV over 1 hour every 12 hours for patients ≥ 40 kg and every 8 hours for patients < 40 kg.
2. Dose adjustments will be made based on toxicity and CSA levels with a targeted trough level of 200-400 ng/mL. Once the subject 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 subjects who are greater than 100% ideal body weight in which case calculation of dose using adjusted body weight is recommended.
4. Subjects 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 Day +180 post-transplant.

B. **Mycophenolate mofetil (MMF)**

1. Mycophenolate mofetil (MMF) will begin on **day 0**, starting approximately 4 - 6 hours after infusion of dilanubicel is completed for subjects receiving **Regimen A or B**.
2. All subjects 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.

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3. Use IV route between days 0 and +7; then, if tolerated, may change to PO beginning on day +8 three times a day.
4. All subjects will remain on MMF 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] ≥ 0.5 x 10^9 /L), whichever day is later, if there is no evidence of acute GVHD and donor CD3 engraftment is at least 50% from the 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.
5. 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).

9.3 Growth Factor

A. All subjects will receive G-CSF 5 mcg/kg/day IV/SC (dose rounded to vial size) starting 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 (unless otherwise directed by the attending physician) 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.

9.4 Supportive Care

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

9.5 Cord Blood and dilanubicel Infusion - See also Protocol Specific Infusion Orders (Fred Hutch)

A. **Unmanipulated cord blood unit (CBU)**

1. The unmanipulated cord blood unit will be infused first.
2. Procedures for requesting, receiving and characterizing the cord blood unit 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 will be according to institutional guidelines.
5. The start and stop time of infusion should be recorded on the infusion record.
6. If the cord blood unit fails to pass inspection or if there is insufficient information to verify the cell product for the subject, notify the Cellular Therapy Lab ((206) 606-1200) and the PI (Filippo Milano, (206) 667-5925, pager (206) 314-1037 immediately).

B. Dilanubicel (Investigational Product)

1. The cryopreserved dilanubicel product is provided in an infusion bag to be thawed and intravenously (IV) infused over approximately 5-10 minutes as clinically possible after any toxicities from infusion of the unmanipulated CBU have resolved. Please refer to Protocol 9910 Receipt and Handling Guidelines for instructions.
2. Please see protocol-specific infusion orders. Infuse product as provided by Cellular Therapy.
3. Infusion time of dilanubicel is approximately 4 hours after **completion** of infusion of the unmanipulated CBU. Variation is allowable for the infusion time of dilanubicel, provided that any toxicities related to infusion of the unmanipulated CBU have resolved. The acceptable

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window of infusion time is 3 hours 30 minutes to 24 hours following completion of the last unmanipulated unit.

4. The subject 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 CBU, repeated dosing is at provider discretion.
5. Supplemental oxygen for standby use should be present in the room.
6. Monitoring of immediate toxicities: See Section 11.3.

9.6 CNS Prophylaxis and Testicular Irradiation

A. All subjects 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 subjects may follow Institutional Guidelines for work-up of potential CNS disease; however, it is strongly recommended that all AML subjects undergo a diagnostic lumbar puncture during pre-transplant evaluation to rule out CNS involvement. For subjects 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 stem cell infusion. Beginning on day +32 post-transplant, subjects may 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 subjects with a low risk of CNS relapse or in subjects who have a high risk of leukoencephalopathy).
D. Testicular irradiation will be performed per standard practice.

9.7 Management of Pre-Engraftment Immune Reactions

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 subjects, 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. Subjects should be monitored carefully for this syndrome.

If subjects 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 subjects 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 subject should be considered to have acute/hyperacute GVHD and should be treated with prolonged steroids as deemed appropriate.

2. For subjects 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. Subjects should be weaned after response as tolerated.

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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

Refer to Fred Hutchinson Cancer Center 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).**

See **Appendix E** for schedule of study evaluations.

In addition to Standard Practice Guidelines complete the following standard care to confirm protocol eligibility:

A. Bone marrow aspirate (and biopsy as clinically indicated), CBC with differential, comprehensive metabolic panel, and creatinine clearance.
B. MUGA or echocardiography (ECHO) 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.
D. Pulmonary function tests; pediatric pre-transplant evaluation qualification: see section 5.1.3, D.
E. Viral screening including CMV PCR (not PCR of patient CMV seronegative).
F. DNA specimen from unmanipulated cord blood unit for chimerism studies (Submitted to Clinical Immunogenetics Laboratory (CIL)).
G. Baseline Panel Reactive Antibody (aka HLA-antibodies) per institutional guidelines.
H. Pregnancy test per Institutional Guidelines in females of childbearing potential.
I. Lymphocyte panel.

10.2 Evaluation on the day of unmanipulated cord blood unit and dilanubicel infusions will include standard assessment as follows (Day 0):

A. Physical exam and review of systems
B. Weight
C. CBC with differential with AM labs
D. Renal and hepatic function: NA, K, CL, CO2, GLU, BUN, CRE, CA, P, ALB, MG, ALT, AST, ALK, BILIT, TP, ALB, LDH

10.3 Evaluation during infusion of dilanubicel

A. Subject must be monitored during infusion as per institutional guidelines.
B. For any changes in cardiac or respiratory status, notify physician.
C. Obtain vital signs per standard of care for cryopreserved hematopoietic progenitor cells (HPCs).

10.4 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) $\geq$ 500/µl for 2 consecutive measurements**.**
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 or as clinically indicated.
F. Chest radiographs as clinically indicated.

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G. Peripheral blood donor chimerism. See Section 10.6 for details.
H. Bone marrow aspirate (+/- biopsy, as clinically indicated) on Day 28 ±7 days for disease restaging (morphology, immunophenotyping, cytogenetics and molecular studies as indicated) and assessment of donor engraftment. Bone marrow specimen, *unsorted*, sent to CIL for chimerism studies.
I. Total T lymphocytes and subset enumeration (See Section 10.8).
J. Blood samples for Immune Reconstitution Studies (See Section 10.8)
K. GVHD evaluation (**Appendix B**) weekly or as clinically indicated.
L. Other tests (e.g., x-rays and tumor markers) as clinically appropriate for assessment of underlying malignancy on Day 28.

10.5 Subject Evaluations from Engraftment through Day 100, 6 Months 1 and 2 Year (please see evaluation time point table for windows +/- under Section 10.10)

A. *Physical examinations at least weekly until discharge, then as clinically indicated during first 100 days, Day 180, 1 and 2 year.
B. Vital signs twice weekly until discharge.
C. Karnofsky/ECOG performance status (**Appendix A**) once at Day 100, Day 180, and 1 year.
D. Complete blood count with leukocyte differential at least weekly and/or as clinically indicated through Day 100 or until discharge then at Day 180, and 1 year.
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 100 +/- 20 days and 1 year for assessment of donor engraftment and disease restaging. Bone marrow aspirate submitted, *unsorted*, to CIL for chimerism studies.
J. Peripheral blood for chimerism studies. See Section 10.6 for details.
K. Quantitative immunoglobulin levels (IgG, IgA, IgM) at Day 56, 100, Day 180, and 1 year.
L. Total T lymphocytes and subset enumeration (See Section 10.8).
M. Blood samples for Immune Reconstitution Studies (See Section 10.8).
N. GVHD evaluation (**Appendix B**) weekly and/or as clinically indicated through Day 100 (or longer if clinically indicated), then at 6 months, 1 year, and 2 year.
O. Panel Reactive Antibody (aka HLA antibodies) once upon count recovery per PI discretion.

* At the 2-year time point, only physical examination to be done.

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

Chimerism studies will be performed as outlined below. Subsequently, if the subject'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).

A. Bone marrow aspirate (unsorted) for chimerism studies at Day 28, 100, and 1 year. After 1 year, bone marrow chimerism is to be performed as clinically indicated.
B. Bone marrow aspirate for morphology and immunophenotyping by flow cytometry at Day 28, 100, and 1 year. After 1 year, bone marrow evaluation is to be performed as clinically indicated.
C. Clinical peripheral blood donor chimerism: Day 21*, 28*, 56*, 100, and 1 year:
Peripheral blood is to be collected and cell sorted for $CD3^+$, $CD33^+$, and $CD56^+$ and donor/host chimerism determined (only on unmanipulated cord blood unit). Additional timepoints for

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peripheral blood chimerism may be collected as clinically indicated per the attending physician. If performed, these data will be collected.

D. **Only for research**: Peripheral blood donor chimerism: Day 7 and 14 peripheral blood is to be collected for donor/host chimerism studies.

* Day +21, 28, and +56 chimerism may be skipped IF the patient had prior chimerisms and it showed at least 95% donor engraftment from the single cord blood unit.

10.7 Residual/Recurrent Disease Evaluation

A. Subjects will be evaluated routinely for evidence of recurrent malignancy as per Institutional Guidelines. If at any time the attending physician suspects recurrent disease, additional analyses will be performed as clinically indicated.

10.8 Immune Reconstitution Clinical Studies

A. Quantitative immunoglobulin levels (IgG, IgA, IgM) will be assessed at pre-conditioning, Day 28, 56, and 100 (per institutional practice), and as possible at Day 180, 1 year.
B. Total T lymphocytes and subset enumeration (lymphocyte panel) will be performed prior to conditioning, Day 28, 56, 100, and as possible at Day 180, 1 year.
C. **Research:** As possible, an additional 5 mL peripheral blood sample will be collected and sent for lymphocyte subset analysis for T, B and NK cell subsets in patients beginning on ~ day +7 (or if/when there is an observed spike in lymphocyte counts) for a maximum of 5 consecutive days following the observed spike in lymphocyte counts. This will be drawn with AM labs.

10.9 Graft vs. Graft Studies

A. Graft vs. Graft (a.k.a Host-Donor Immune Interaction) studies will be conducted *as possible* on all patients to gain a better understanding of the immune interaction of the host vs donor and donor vs donor, and the impact of these interactions on clinical outcomes such as the kinetics of hematopoietic reconstitution and immune recovery and the potential for immunomodulatory effects directed by dilanubicel.
    - Assays for immune mediated responses occurring between the host and donors, including cytokine analysis: approximately 30 mL of peripheral blood will be collected *as possible;* prior to the start of conditioning, and on day 3 (±1 day), 7 (±2 day), 14 (±3 days), and 28 (±7 days). The samples collected at day 7 and 14 will be also used for chimerism analysis per Section 10.6 (D). Send samples to the Hadland Lab. Samples should be collected MONDAY through FRIDAY ONLY.

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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 Time point Post Transplant	Window
Day +3	± 1 day
Day +7	± 2 days
Day +14	± 3 days
Day +21	± 3 days
Day +28	± 7 days
Day +56	± 7 days
Day +100	± 20 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 subject's circumstances at these time points.

10.11 Subject Withdrawal or Off-Study Criteria

A subject may be withdrawn from the study for any of the following reasons:

- Subject or legally authorized representative withdrawal of consent
- Subject determined ineligible by the study team after consent/initial enrollment
- Study discontinuation as determined by the IND Sponsor (Medical Monitor) and/or the PI
- Lost to follow-up
- Death

The subject is no longer followed, and no study procedures and assessments are performed after the subject was considered to have met the above-noted criteria. Previously collected data prior to withdraw may be reviewed by the study team.

Partial withdrawal:
A subject may decline or withdraw from research sample collection(s) but allow the study team to continue follow up of associated clinical outcome information. The date and reason for subject withdrawal will be recorded in the case report forms and discussion will be recorded and documented in the medical records.

All subjects who withdraw with an ongoing AE will be followed until resolution of the event, death, or until the PI determines that the event is stable. If a subject is lost to follow-up, information will be recorded and documented in the case report form.

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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

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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 dilanubicel

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. Dilanubicel (Please refer to Section 9.5 for infusion guidelines)

- 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

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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 $\geq$ 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. If the patient does not respond adequately to supportive care alone, methylprednisolone will be administered at 2mg/kg IV every 12 hours for 2 total doses.

- Delayed toxicities: It is possible that infusion of dilanubicel 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

Nephrotoxicity	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

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responsible for the abnormality. These adjustments should be discussed with the PI. Dose adjustments are described in Section 9.2.

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 DEFINITIONS

A. Primary Graft Failure: Patients will be considered primary graft failure/rejection provided they meet any criteria listed below:
   1. No neutrophil recovery by day 45 (regardless of donor chimerism)
   2. No autologous recovery (neutrophil recovery but < 10% donor chimerism in blood and BM) by day 45.

B. The day of neutrophil recovery will be the 1st day of 2 consecutive days of absolute neutrophil count at or above 500 after the 1st post-CBT nadir.

C. Platelet engraftment will be defined as the first of a platelet count > 20,000/µl without subsequent transfusions for 7 days.

D. 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).

E. GVHD will be graded according to **Appendix B**.

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

13.0 GUIDELINES FOR ADVERSE EVENT REPORTING

13.1 Adverse Events

13.1.1 Definitions

a. Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

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b. Serious Adverse Event

A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:

- Death.
- Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
- In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization. Additionally, hospital admissions for administration of the study drug, procedures required by the study protocol, planned elective procedures, or tumor-related diagnostic procedures are not considered SAEs.
- Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect.
- An important medical event that requires intervention to prevent one of the above outcomes.

c. Unexpected Adverse Event

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure. “Unexpected,” as used in this definition, refers to an adverse drug experience that has not been previously observed and reported rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

d. Suspected and unexpected serious adverse reaction (SUSAR):

As described in 21 CFR 312.32, a serious adverse reaction is considered unexpected if it is not listed in the investigator brochure or it is not listed at the specificity or severity that has been observed. Unexpected, as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

13.1.2 Monitoring and recording AEs

Any medical condition already present at the time of consent should not be reported as an AE unless the medical condition (or signs or symptoms) present at informed consent changes in severity, seriousness, or frequency of occurrence at any time during the study. In this case, it should be reported as an AE.

All adverse events will be assessed by the investigator or qualified designee and recorded in the CRFs. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug or a study procedure

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- The action taken due to the adverse event
- The outcome of the adverse event

When an adverse event increases in severity, the event will be captured at its highest grade.

Patients enrolled in this study are receiving myeloablative pre-transplant treatments and other transplant related procedures that are generally associated with high rates of anticipated adverse events (refer to **Appendix C**).

The following events will not be collected 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.
- 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 one or more of the following criteria are met:
    - Any clinically significant result that is not part of another reported clinical diagnosis
    - Any result that meets the definition of an SAE
    - Any result leading to discontinuation or interruption of dilanubicel
    - Any result that requires therapeutic intervention or a change in subject management

  Laboratory abnormalities that do not meet the above conditions will not be recorded on the CRF.

13.1.3 Grading of the severity of an Adverse Event

All AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event.

13.1.4 Attribution of Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

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- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent, but could be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

13.1.5 Adverse Event Reporting Period

Grade ≥ 3 adverse events (or highly unusual grade 2 adverse events) and all serious AEs (SAEs), regardless of relationship to dilanubicel or transplant, will be collected from the start of study treatment (pre-transplant conditioning) until 100 days following transplant. After day 100 patients will be tracked for progression and survival as well as secondary endpoints. After the 100 days following transplant through the end of the subject's study participation, only those AEs considered by the investigator to be related to dilanubicel will be recorded. Any ongoing dilanubicel-related adverse events and/or serious adverse events should continue to be followed until they have resolved or become stable. A subject withdrawn from the study because of an adverse event should be followed until the clinical outcome from the adverse event is determined.

If a patient experiences relapse or graft failure and goes on to further treatment off protocol, only those AEs considered by the investigator to be related to dilanubicel will be recorded in addition to death. Adverse events will be recorded in the subject's medical record and in the CRF. Each AE will be evaluated for duration (onset and resolution dates), severity, outcome, seriousness, and causal relationship to dilanubicel and/or transplant.

Recording Diagnosis versus Signs and Symptoms – Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Investigator and recorded in the CRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, it should be recorded as a separate AE in the CRF. Additionally, a condition that leads to a medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, or transfusion) should be recorded as an AE rather than the procedure.

13.2 Adverse Event Reporting Requirements

13.2.1 IRB Reporting

The principal investigator must report adverse events which in his opinion are unexpected, related or possibly related to the investigational product, 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, in an expedited manner. For SAE reporting, refer to Fred Hutch IRB institutional policies. SAEs and deaths not meeting expedited reporting criteria will be reported to the IRB as part of the annual continuation review report to the IRB, or as otherwise directed by IRB policy.

The principal investigator must 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 (relative to the IRB-approved protocol, terms of IRB approval, or regulations governing the conduct of human subjects research) in accordance with Fred Hutch IRB institutional policies.

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The principal investigator will be requested to provide written notification of IND safety reports issued by the Sponsor to the IRB of record as soon as is practical, consistent with IRB requirements.

In accordance with Fred Hutch institutional policy, AEs which in the opinion of the principal investigator meet all three of the following criteria will be reported to the IRB after he or she first becomes aware of the problem. Expedited reporting criteria:

1. unexpected with regard to what is known about the investigational product
2. related or possibly related to the investigational product
3. 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

AEs 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.

13.2.2 Sponsor Reporting

Classification of an event as serious or non-serious (see Section 13.1) determines the reporting procedures to be followed by the research site for reporting the event to the Sponsor, as summarized in the Table below:

Classification		**Reporting Time**	**Reporting Action**	**IND Sponsor Contact Information**
Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team awareness	Email notification to IND Sponsor	ISIOC email: ISIOC@fredhutch.org
	All SAEs	Within 2 business days of research team awareness	Submit completed Institution-Sponsored IND SAE Reporting Form signed by PI or designated sub-Investigator	ISIOC email: ISIOC@fredhutch.org
Non-serious AE		Per CRF completion guidelines	Record on appropriate CRFs	N/A

[*] *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 physicians 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.*

The information in the Institution-Sponsored IND SAE Reporting Form must match or be reconciled with the information recorded in the adverse events section of the CRF and study database. For example, the same adverse event term should be used on both forms. The report must include the date, severity and duration of the event, the relationship, 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.

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 serious adverse event report received from the Fred Hutch PI will be evaluated by the Medical Monitor, who will assess the seriousness of the event (see Section

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13.1), the expectedness of the event (see Section 13.1), and the relationship to participation in the study (see Section 13.1). For regulatory reporting purposes, the Sponsor will determine expectedness relating to the investigational product using safety information specified in the investigator brochure. An event will be classified as related if either the investigator or the Sponsor determines that the event may be related to the study agent.

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

The Sponsor or its designee will provide all investigators with a safety letter notifying them of an event that meets FDA IND Safety Reporting criteria. Investigators will be requested to provide written notification of safety report to the IRB of record as soon as is practical, consistent with IRB requirements.

NHLBI reporting

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).

14.0 DATA AND SAFETY MONITORING PLAN

Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, Fred Hutch Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), Fred Hutch Scientific Review Committee (SRC) and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines, study agreements, and ICH Good Clinical Practices.

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 Fred Hutch 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.

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15.0 DATA MANAGEMENT/CONFIDENTIALITY

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique subject number to protect 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.

Data will be entered into the Fred Hutch research database for the following time points: approximately day + 28, +56, +84, +365, and +730 post-transplant. Primary source documents regarding patient outcomes are collected to allow verification of protocol compliance, data accuracy and completeness and full and timely reporting of safety data. The study staff will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion.

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	5	6	11
Not Hispanic or Latino	12	12	24
Ethnic Category Total of All Subjects*	17	18	35
Racial Categories			
American Indian / Alaska Native	1	2	3
Asian	5	5	10
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	2	2	4
White	9	9	18
Racial Categories: Total of All Subjects*	17	18	35

The primary objective of this study is to assess the safety of infusing dilanubicel as **a short-term graft source** in the context of single donor CBT. The endpoints to be used for this purpose include **graft failure and severe GVHD**. 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. To that end, we have implemented stopping rules for each of these complications.

A total of 35 participants will receive the investigational product on this study, and the study will be suspended pending further review if, at any time, there is sufficient evidence to suggest that the true probability of graft failure exceeds 15%, or the true probability of severe GVHD exceeds 25%. Sufficient evidence will be taken to be an observed failure rate whose lower one-sided 80% confidence limit exceeds the thresholds listed above (25% for GVHD, 15% for graft failure). Operationally, these limits will be met if any of the following is observed.

Grade 3-4 acute GVHD: 2/2-3, 3/4-6, 4/7-9, 5/10-12, 6/13-16, 7/17-19, 8/20-23, 9/24-26, 10/27-30, 11/31-34

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Graft Failure: 2/2-5, 3/6-10, 4/11-15, 5/16-21, 6/22-26, 7/27-32, 8/33-34

The table below summarizes the probability of each of these limits being met (last 2 columns of table) at or before the 10th, 20th and 30th patients are studied, under a variety of assumed-true failure probabilities (2nd column of table).

Number Pts	True Prob of Failure	Grade ≥4 Acute GVHD	Graft Failure
10	0.05	0.01	0.03
20	0.05	0.01	0.03
30	0.05	0.01	0.03
10	0.10	0.04	0.11
20	0.10	0.04	0.14
30	0.10	0.04	0.13
10	0.25	0.26	0.54
20	0.25	0.35	0.64
30	0.25	0.38	0.63
10	0.30	0.38	0.67
20	0.30	0.51	0.78
30	0.30	0.57	0.77
10	0.40	0.86	0.85
20	0.40	0.85	0.92
30	0.40	0.88	0.93

If any of the above stopping rules are met at any time, the study will be suspended pending review by the DSMB for a recommendation regarding termination or continuation of the trial. In addition, we shall carefully monitor time to engraftment. And while no formal “stopping rule” will be set for this endpoint, should the average time to engraftment among patients treated on this study appear to be longer than that in an appropriate historical control group, the study will also be suspended pending a review as described above. In general, we expect this procedure to result in a faster time to engraftment, so a rough guideline for triggering concern in this regard would be an average time that is longer than that in the historical control group (25 days).

17.0 TERMINATION OF STUDY

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

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18.0 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

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Appendix B 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*
	☐	+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

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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	≥ 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/m2/day
2	1000 – 1499 mL/day diarrhea	556-833 mL/m2/day diarrhea
3	≥ 1500 mL/day diarrhea	>833 mL/m2/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

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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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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 $\geq$ 3x upper limit of normal, AST or ALT $\geq$4x upper limit of normal or total serum bilirubin $\geq$ 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 Fred Hutch

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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 Fred Hutch

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Appendix C Potential Adverse Events Associated or Expected with HCT

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.

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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 ventricular 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 accident	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 rheumatic	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_1 >65%-80% or Dyspnea 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 Karnofsky 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 aminotransferase; ALT, alanine aminotransferase

Appendix E Schedule of Study Evaluations

	Screening	Prior to Conditioning	Days Post-transplant									Long Term Follow-up	
			0	Per Standard of Care until Engraftment	3 ± 1 day	7 ± 2 days	14 ± 3 days	21 ± 3 days	28 ± 7 days	56 ± 7 days	100 ± 20 days	180 ± 30 days	1 and 2 years
Informed Consent	X												
Demographics	X												
Medical History	X												
Physical Exam	X		X	X							X	X	X
Performance Status	X										X	X	X
Pregnancy Test	X												
Metabolic Panel	X		X	X									
Creatinine Clearance	X												
MUGA or ECHO	X												
Pulmonary Function Tests	X												
Disease Evaluation	X								X		X		X
Chest CT	X												
CBC w/ differential		X	X	X								X	X
Patient and CBU DNA specimen for chimerism		X											
Vital Signs			X	X									
Weight	X		X										
dilanubicel Infusion			X										
GVHD Evaluation											X	X	X
Adverse Events		X[F]	Continuous									X[G]	X[G]
Panel Reactive Antibody		X	Once upon count recovery										
Bone Marrow Chimerism - Clinical									X		X		X[C]
Peripheral Blood Chimerism – Clinical								X[B]	X[B]	X[B]	X		X
Peripheral Blood Chimerism – Research						X[D]	X[D]						
Immune Reconstitution													
Immunoglobulin levels (IgG, IgA, IgM)		X							X	X	X	X[A]	X[A]
Lymphocyte Panel		X				X[E]			X	X	X	X[A]	X[A]
Graft vs. Graft													
Host-Donor Immune Interaction		X			X	X[D]	X[D]		X				

[A] As possible at D180 and 1 year. Not required at 2 years post-transplant.
[B] Subsequent chimerisms may be skipped IF the patient had a prior chimerism that showed at least >95% donor engraftment from a single cord blood unit and with PI approval.

[C] After 1 year, bone marrow chimerism is to be performed as clinically indicated.
[D] On day +7 and +14: 30 mL of peripheral blood will be drawn once to be used for both donor/host chimerism research studies and assays for immune mediated responses.
[E] Starting on day +7 labs will be assessed for lymphocyte spike and a lymphocyte panel will be drawn for 5 consecutive days following observed spike for subset analysis.
[F] Adverse event assessment will begin at the start of conditioning.
[G] Only those AEs considered by the investigator to be related to dilanubicel will be recorded.