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FRED HUTCHINSON CANCER CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

Title:	Infusion of off-the-shelf ex vivo expanded cryopreserved progenitor cells to facilitate the engraftment of a single CCR5Δ32 homozygous or heterozygous cord blood unit in patients with HIV and hematological malignancies
Protocol Number:	1004070
Current Version:	September 2, 2022
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IND Number:	19254
Investigational Agent:	Non-matched off-the-shelf (OTS) ex vivo expanded cryopreserved progenitor cells (dilanubicel)
Study Regimen:	Regimen A: High dose consisting of 1320 cGy TBI plus fludarabine plus cyclophosphamide followed by unmanipulated cord blood and dilanubicel Regimen B: Intermediate dose consisting of 400 cGy TBI plus Fludarabine plus cyclophosphamide plus thiotepa followed by unmanipulated cord blood and dilanubicel
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Signature Page:

Investigator Statement:

I have carefully read Protocol 1004070 entitled *Infusion of off-the-shelf ex vivo expanded cryopreserved progenitor cells to facilitate the engraftment of a single CCR5Δ32 homozygous or heterozygous cord blood unit in patients with HIV and hematological malignancies* version date September 2, 2022.

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): _____

Institution and Address: _____

Phone Number: _____

Signature: _____Date: _____

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This is a Phase II prospective, open label, multi-site, non-randomized study in which patients with HIV and hematological malignancies will be enrolled. This trial will focus on evaluating the safety of single unit cord blood transplant in patients with hematological malignancies and HIV infection in need of allogeneic stem cell transplant (alloSCT) when the non-matched off-the-shelf (OTS) ex vivo expanded cryopreserved progenitor cells are infused. Throughout this protocol, the investigational product will be referred to as dilanubicel.

Patients undergoing a cord blood transplant (CBT), who are often of minority or mixed ethnicity background, are at increased risk of infection and early death following the transplant due to the significant delay in white blood cells recovery (in particular, neutrophils) that these patients experience. With the goal of overcoming the significant delay in neutrophil recovery that occurs following transplantation with umbilical cord blood (UCB), 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. To this end, 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 that is safe and resulted in significantly earlier neutrophil engraftment post myeloablative CBT [1]. While the first clinical trial utilized expanded CD34+ cells isolated from a UCB donor selected on the basis of HLA-matching, more recent studies have used this expanded product as an "off-the-shelf" (OTS) universal donor in the setting of a standard of care double CBT. Based on our results to date, we now propose to eliminate the need for a second UCB unit by using our expanded dilanubicel cell product to provide the initial wave of early hematopoietic recovery until the long-term HLA matched donor engrafts. This approach would overcome the cell dose barrier and delayed hematopoietic recovery and may also reduce the incidence of acute graft versus host disease (GVHD), which is higher in the setting of double unit CBT [2]. The goal is to reduce early transplant related mortality while taking advantage of the powerful anti-leukemia effect in CBT thereby leading to improved long-term survival.

2.0 BACKGROUND

2.1 Study Disease

AlloSCT has the potential for elimination of the HIV reservoir: AlloSCT is a well-established procedure for treatment of life-threatening malignant and nonmalignant hematologic disorders [3]. Its success is based on the capability of the donor graft immune cells to eliminate recipient hematopoietic cells during establishment of full donor chimerism, as well as donor primitive hematopoietic stem cell (HSC) ability to self-renewal and differentiate, characteristics that allow transplantation of small numbers of HSC sufficient for complete replacement of the recipient hematopoietic and immune system. Allogeneic donor HSC ultimately will differentiate into multiple hematopoietic lineages, including erythrocyte, monocyte/macrophage, granulocyte, megakaryocyte, and lymphoid cells. Cure of hematologic disorders is mediated by allogeneic donor immune clearance of all recipient hematopoietic cells, a process termed 'graft versus leukemia' [4]. Establishing a new donor T cell compartment that is resistant to HIV infection is the rationale of using alloSCT to cure HIV. Unfortunately, the majority of alloSCT protocols carry exclusion criteria for patients with known HIV infection. HIV exclusion in current alloSCT protocols is reflective of early concerns as to its safety in this patient population. However, recent studies demonstrate the safety and efficacy of alloSCT in HIV-infected patients [5]. HIV treatment with antiretroviral therapy (ART) over the past 30 years has transformed this life-threatening viral infection to chronic suppressive therapy. However, HIV infection is associated with an increased risk of hematologic malignancies over time, particularly Hodgkin and non-Hodgkin lymphoma [6]. Lymphomas in HIV-infected patients are in general more aggressive with observed higher relapse rates and lower survival compared to patients who are not HIV-infected. Taken together, there is an unmet medical need for HIV-infected patients with hematologic malignancies for whom alloSCT is indicated, with dual therapeutic goal to attain allogeneic-mediated control of their hematologic disease, improve their survival, and eliminate their HIV reservoir.

The only well-established cure of HIV was accomplished by transplantation of an HLA-matched unrelated allogeneic graft from an adult donor who was homozygous for the CCR5Δ32 allele [7]. Although ART was stopped before myeloablative conditioning, serum levels of HIV-1 RNA have remained undetectable for the ensuing 9 years of follow-up. Proviral DNA was detected on day 20 and 61 after transplant, but not thereafter.

Although HIV-specific T cell responses were detected before alloSCT, these were not detected after transplantation, and the level of HIV-specific antibodies waned over time. This experience suggests that alloSCT has the potential to reconstitute HIV-resistant CD4 T cells, which prevent HIV reactivation through alternative mechanisms over and above immunologic control. Cure has been postulated to have been achieved in two patients transplanted with allogeneic grafts that were not CCR5Δ32 allele homozygous. These patients were conditioned with a reduced intensity conditioning (RIC) regimen that by itself was not sufficient to fully eliminate recipient hematopoietic cells [8]. Therefore, donor chimerism was established primarily through a graft vs. host (GVH) mechanism. In these cases, ART was continued for several years after attainment of full donor engraftment. Preliminary information with 4-year follow up indicates both patients have successfully stopped ART without development of recurrent detectable circulating HIV RNA. These cases support the need to further investigate the mechanisms underlying the ability of alloSCT to cure HIV, whether through establishment of an HIV-resistant graft or by elimination of all host hematopoietic cells including lymphocytes and tissue macrophages through a GVH reaction.

There is an unmet medical need for patients who require alloSCT for management of their malignancy who are HIV infected as current alloSCT trials generally exclude these patients. In our experience ART can be given without interruption after transplantation, controls viral replication, and does not interfere with management of immunosuppressive drugs used to control GVHD [9].

Improbability of finding a CCR5Δ32 homozygous allogeneic marrow donor: If HIV-resistant cells are required to prevent reinfection or cross infection of latent pool, then it becomes important to know if it is biologically plausible to rely on naturally resistant, CCR5Δ32 homozygous, donors. A retrospective analysis performed in our institution of 1273 donors determined that 9 (0.7%) were homozygous for CCR5Δ32 deletion. This result is consistent with studies in the HIV-infected population, wherein the frequency of the non-functional mutant allele CCR5Δ32 (heterozygous expression) is approximately 10% [10]. The low frequency of CCR5Δ32 homozygous individuals must be considered together with the probability of identifying an HLA-matched adult donor. Within the volunteer unrelated adult registries, the chances for a donor matched for HLA-A, B, C, and DRB1 alleles depend on recipient ethnicity, specifically 57% for Caucasians, 34% for Hispanics, 27% for Asian-Pacific Islanders, and 18% for African Americans [11]. Thus, the practical reality is that the feasibility to identify an HLA-matched homozygous CCR5Δ32 adult donor ranges only 0.1-0.4% for HIV infected hematology patients in need of alloSCT.

The availability of umbilical cord blood (UCB) expands the number of suitable allogeneic HSC: UCB over the past 30 years has been established as a safe and efficacious graft source for alloSCT, with a global inventory of >650,000 grafts and more than 40,000 transplant procedures performed to date [12]. The immature phenotype of HSC contained in UCB confers a number of distinct advantages, for example a high proliferative capacity. T lymphocytes in UCB exhibit antigen-specificity, [13-16] and are relatively less alloreactive compared to adult allogeneic graft T cells. Indeed, multiple studies have shown that the risk for GVHD following UCB grafting is significantly lower compared to adult marrow or peripheral blood stem cell grafts [17-19]. These characteristics allow allogeneic transplantation with a larger degree of HLA-mismatch than would otherwise be tolerated with an adult-derived marrow or mobilized peripheral blood stem cell graft and without requirement for T depletion as is needed with haploidentical graft alloSCT via administration of cyclophosphamide post-transplant. Thus, the probability of identifying a suitably HLA matched UCB graft given the current global cord blood bank inventory accessible by online registry searches increases to 75% for African Americans, 85% for Hispanics, and >90% for Caucasians or Asian-Pacific Islanders. Recognizing the benefit of UCB in HIV infected patients, UCB banks are recently prospectively identifying UCB grafts from CCR5Δ32 homozygous/heterozygous infants. Recent analysis by the National Marrow Donor Program (NMDP) has included estimates regarding the probability of identifying an adequately HLA-matched UCB unit with a higher cell dose than is required in this study, e.g. $>2.5x10^7$ total nucleated cells/kg (TNC) - to be 73.6% of pediatric patients and 27.9% adult patients. 180 homozygous CCR5Δ32 units were recently identified in the cord blood bank Stemcyte's inventory of 20,000 UCB confirming an incidence of 0.8% [20]. In this proposal, donor search for each enrolled patient includes the approximate 650,000 global inventories available for NMDP online search containing 5,200 homozygous CCR5Δ32 units based on an incidence of 0.8%. Preliminary UCB searches generally identify 15-25 potential grafts matched at 4 of 6 HLA loci or better and containing varying total nucleated cells (TNC). As administration of a second ex vivo

expanded UCB could allow safe and effective engraftment of UCB containing a lower cell dose, e.g. >1.5x10^7/kg, the likelihood of identifying homozygous CCR5Δ32 for any patient enrolled on this protocol is expectedly higher than 73.6% of pediatric and 27.9% of adult subjects [21].

The limitation of single unit UCB transplantation is the number of HSC within banked units: The number of HSC within a UCB unit, whether measured by TNC, CD34+ cells, or colony-forming cells, is directly correlated with the probability of engraftment, as well as transplant related mortality (TRM) [22,23]. Analysis of adult single UCB transplantation (SCBT) recipients demonstrated that infused CD34+ cell dose is the most important predictor of myeloid engraftment, consistent with results from multiple other studies in pediatric recipients [24]. In order to overcome the limitation of cell dose, the procedure of infusing two units, termed "double cord blood transplant (DCBT)" has been developed. The rate of graft rejection after DCBT is lower than expected after transplantation of a single unit of similar size [24]. While infusion of two units appears to be important to prevent graft rejection, in the majority of DCBT procedures, only one of the UCB units engrafts long-term [1]. The "non-engrafting" unit is thought to facilitate engraftment through yet undefined mechanisms that may include graft-graft immune reactivity. Unfortunately, at the current time, there is no ability to reliably pre-determine which unit will engraft long-term after DCBT [24]. In this background challenge, 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 UCB units which could eliminate the need for transplantation of two un-manipulated UCB grafts. As UCB units expanded in Notch ligand condition do not contain any T cells, the un-manipulated UCB graft uniformly engrafts long-term, while the Notch ligand expanded cells with rare exceptions only persist in the patient for a few weeks. Results from our 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 is observed after conventional UCB [1,2].

Stem Cell Transplant for Treatment of Malignancy in HIV-1-Infected Individuals: Important questions related to transplant in HIV-1-infected individuals include whether there are enough HIV-1-infected children and adults who require transplant for treatment of an underlying disease to perform this study and, if so, is there data supporting transplantation in individuals with HIV-1 infection. The answer to both questions is yes. With the improved life expectancy of HIV-1 infected persons treated with highly active antiretroviral therapy (HAART), the rate of malignancies has increased; in particular, that of B-cell Non-Hodgkin lymphoma, which may be treated with autologous or allogeneic stem cell transplantation. In addition, the Surveillance, Epidemiology and End Results (SEER) data demonstrate a significantly higher incidence of several cancers that are treated with allogeneic hematopoietic cell transplantation in HIV- infected individuals compared to similar aged individuals without HIV-1, including Hodgkin lymphoma and leukemia [25]. In a review of the CIBMTR registry data, 23 participants with HIV-1 infection were identified as having undergone allogeneic transplant [26]. Nine of the 23 (39%) underwent allogeneic hematopoietic cell transplantation after 1996, in the HAART era. At a median follow-up of 59 months, six of the 23 participants were alive. Four of the nine participants transplanted after 1996 were alive whereas only 2 of 14 pre-1996 participants were alive. The data suggest that allogeneic hematopoietic cell transplantation is feasible for selected HIV-1-positive participants.

A complicating factor in the management of HIV-1 infection in individuals who require hematopoietic cell transplantation is the ability to continue HAART in setting of hematopoietic cell transplantation due to their inability to take the medications, as well as drug-drug interactions. This can be managed with reduced intensity allogeneic hematopoietic cell transplantation. Reduced-intensity conditioning aims to suppress the participant's immune system sufficiently so that it will accept the donor stem cells and is especially helpful for older adults or less clinically fit participants who may be poor candidates for myeloablative conditioning regimens. There is growing interest in using reduced intensity conditioning (RIC) prior to potentially curative allogeneic hematopoietic stem cell transplantation transplant. Reduced-intensity allogeneic HCT for participants with HIV-1 has expanded with the recent publication of the Ohio State University experience [27]. In this report, three subjects (ages39-55 years) with HIV-1 underwent allogeneic hematopoietic cell transplantation. The underlying malignancies included acute myeloid lymphoma, Burkitt lymphoma and plasmablastic lymphoma. Conditioning consisted of fludarabine and busulfan with or without antithymocyte globulin (1 subject without). Graft versus host disease prophylaxis included tacrolimus and mini-dose methotrexate. Subjects

continued HAART throughout the transplant course without interruption. One subject developed acute graft versus host disease. At the time of the study publication, all subjects were alive, free of the malignant disease and off immunosuppressive therapy 368 to 802 days post-transplant [28].

High-dose chemotherapy with autologous hematopoietic cell transplantation has been extended to participants with AIDS- related non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma and results appear to be comparable to those achieved in participants without HIV-1 infection. Across multiple trials, the probability of survival after high-dose conditioning followed by autologous hematopoietic cell transplantation for participants with AIDS-related NHL ranges between 39% and 85% [29]. The incidence of malignancy or other disorders requiring hematopoietic stem cell transplantation in HIV-1 infected children is low, but it does occur. In a recent survey (May 2011) of 19 domestic International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) sites, the sites reported 24 HIV-1 infected children or adolescents with a malignancy in the last 10 years. The diagnoses include acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), lymphoma and Burkitt's lymphoma. Ages range from one year to 20 years, with the majority in the 8 to 10-year-old age group.

A recent study on two HIV-1 infected individuals (including a perinatally-infected young adult) each with a malignancy, reported no detectable HIV-1 proviral DNA, single copy HIV-1 plasma RNA or replication-competent CD4 T cell reservoirs post-transplant. Both individuals received a reduced intensity allogeneic hematopoietic cell transplantation (not CCR5 deleted) and were continued on HAART. These patients, though originally CCR5Δ32 heterozygous, are now wild type with complete engraftment of donor cells. However, they are still receiving HAART and therefore are not considered cured of the infection [30]. Furthermore, studies of tissue reservoirs have not been performed. These data suggest that the replacement of the recipient's cells with donor hematopoietic cell transplantation (even with potentially susceptible cells) under treatment with HAART may have a significant effect on persistence of HIV-1 in patient reservoirs.

As noted previously, double cord transplants are used in adults to enhance engraftment and myeloid and platelet reconstitution and increase the cell dose although by 100 days, only one of cords predominates and fully engrafts [31]. In an HIV-infected individual, it is possible that a CCR5 Δ 32 homozygous cord unit will have a selective advantage over the non-deleted unit since it is innately resistant to HIV-1 infection. Together, these results indicate that although uncommon, there are HIV-1infected adults and children who will require stem cell transplantation for treatment of a malignancy and could potentially benefit from the availability of a cord blood unit donor bank and would be available for enrollment into this protocol.

Our team anticipates that UCBT with CCR5Δ32 cells may lead to continued suppression of HIV-1 infection without the necessity of antiretroviral therapy. The choice of discontinuing HAART will not be dictated by the protocol; however, if the patient and his/her clinical care providers opt to interrupt HAARTs, this will be of extreme interest to the protocol team. Upon discontinuation of HAART for 14 or more consecutive days once the patient is greater than 100 days post-transplant, a modified follow-up schedule of evaluations will begin, which will assess virologic control and immune health off HAARTs, and potential for cure. The team anticipates that over 8 to 12 months post-transplant viral reservoirs may diminish as the donor monocytic and lymphocytic lineage CCR5Δ32 homozygous cells engraft. This strategy will provide proof of concept for future advances in stem cell and gene therapy promoting HIV-1 resistant cells as immunotherapy for HIV-1.

2.2 Preclinical Data

Overview of Preclinical Studies Notch-based UCB ex vivo expansion: UCB is selected as a graft source in this pilot study due to its tolerance of HLA mismatch without requirement of T cell depletion. However, UCB low cell dose can be a limitation in rates and kinetics of engraftment. With this in mind, we will provide dilanubicel cellular product along with the identified HIV resistant UCB graft to ensure rapid and consistent full donor engraftment. Throughout the preclinical development of the dilanubicel cellular 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. 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.

In vivo repopulating ability is retained following cryopreservation of the expanded cell product: Herein by successful multi-lineage engraftment in an immunodeficient xenograft mouse model we demonstrate that cryopreserved ex vivo expanded cellular therapy can be thawed and infused without any loss of ability to repopulate. As shown in **Figure 1**, 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. Additional experiments confirmed that repopulating ability of the expanded cell product is retained following cryopreservation. All mice that received expanded cryopreserved cells engrafted (defined >0.5% human CD45 in the marrow) with an average overall human engraftment of 8% at 2 weeks post infusion and 7% at 4 weeks post infusion.

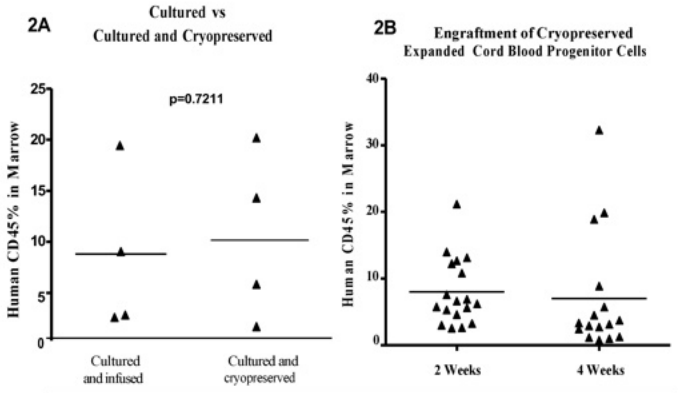


Figure 1: In vivo repopulating ability of cultured, cryopreserved cells.

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 showed 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 (IR) 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 [32].

GvH responses reactivate latently HIV-infected cells which could become target to NK cells: Activation of quiescent retroviruses, such as murine leukemia viruses in mice, after graft-induced allogeneic disease (GvHD) has been described [33-35]. Although mixing of allogeneic PBMCs has also been demonstrated to activate resting CD4+ T cells in vitro and to stimulate retroviral transcription and translation, [36, 37] in vivo graft-versus-host effects also reactivates integrated retroviruses. For example, all patients in the UCSF cohort analyzed thus far experienced low level, but detectable viremia or increased cell-associated HIV RNA following reduced intensity allogeneic HSCT in the setting of suppressive ART as detailed below. GvHD is a result, in part, of release of inflammatory cytokines [38] which may explain the increased release of virus from PBMCs observed in our patients. In addition, non-human primate studies revealed that CD8+ T cell depletion can lead to loss of HIV control, even in the setting of previously suppressive ART [39]. Gut microbial translocation and TLR activation in the setting of transplant and GvHD disease of mucosa is also possible. Regardless of the specific mechanisms, reactivation of HIV reservoirs with subsequent NK responses to these reactivated, "altered self" cells may lead to specific anti-HIV effects. This mechanism could trigger a significant decay of the HIV reservoir by activated NK cells.

Cell-associated HIV-1 DNA decreases following allogeneic HSCT in the setting of HIV reactivation:
We report on 3 additional HIV-1-infected individuals on suppressive combination antiretroviral therapy (cART) who underwent allogeneic HSCT for hematologic malignancies (Participants C, D, E). All patients were on cART with suppressed viremia prior to HSCT and underwent either reduced-intensity conditioning alloSCT for lymphoma (C and D) or fully myeloablative alloSCT for leukemia (E). All experienced rapid decay in HIV-1 DNA in PBMCs and CD4+ T cells following HSCT, with significant decreases observed prior to the development of full donor chimerism in some individuals. All of our patients analyzed to date experienced either low level viremia or increased HIV transcription despite combination ART and rapidly declining CD4+ T cell HIV DNA levels in the months following HSCT (**Figure 2**).

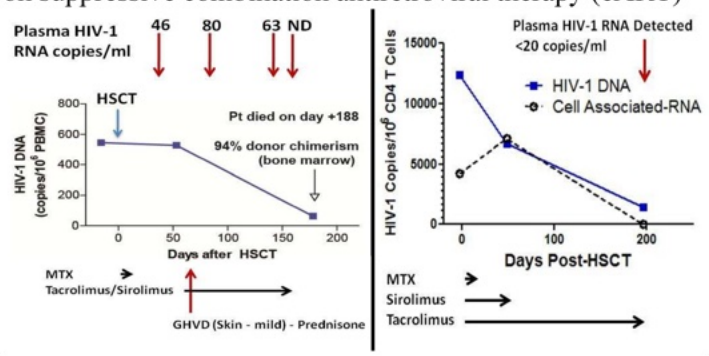


Figure 2. Relationship between HIV-1 DNA decay: cell-associated DNA, low level viremia, and GVHD following alloSCT in Boston patients C (A) and D (B). Patient E also experienced a reduction in HIV-1 DNA following myeloablative alloSCT.

Correlates of reservoir decay following infusion of CCR5 modified CD4 T cells: We monitored correlates and mechanisms of immune reconstitution and HIV reservoir decay in two independent pilot clinical trials initiated by Sangamo BioSciences, in which autologous CCR5-modified CD4 T-cells (SB-728-T) generated by transducing enriched CD4 T cells with an adenoviral vector encoding the CCR5-targeting ZFNs were infused in HIV+ immune non-responders (subjects with CD4 T cell counts between 250 and 500 cells/µl). Results generated from the first SB-728-T clinical trial (902 trial) demonstrated that a single infusion of SB-728-T led to sustained increases in CD4+ T cell counts through 3.5 years (33-44 months) compared to baseline (median increase of +193 cells/µl, $p = 0.02$). The degree of long-term immune reconstitution was associated with expansion and persistence of a novel memory stem cell CD4+ T cell population (TSCM). This novel TSCM subset (CD45RAintCD45ROintCCR7+CD27+ CD95+CD58+) was highly enriched in CCR5 modifications (23.2% ± 17.6 modified alleles at 3yrs), confirming that these cells originated from the product. Importantly, these cells had significantly lower levels of integrated HIV-DNA than other memory cells at 3.5 years post-infusion (mean of 172 copies/1e6 cells vs 1133 and 2415 copies/1e6 for central (TCM) and effector (TEM) memory cells), suggesting that these memory stem cells (CD45RAintROint CD95+) are derived from naïve T cells during manufacture and that their long term persistence could dilute the HIV reservoir by a process of differentiation from TSCM to TEM.

Our results showed that SB-728-T infusion led to a significant decrease in the levels of total HIV DNA, as measured in subjects' PBMCs by ddPCR, in 6 out of 9 subjects 12 months post-infusion (from -0.23 to -0.5 log decrease). Interestingly, changes in HIV DNA were higher between BL and long-term follow up (from -0.69 to -3.6 log decrease) than between BL and month 12, demonstrating that the decrease in the size of the HIV reservoir is an on-going process and hence cannot be solely explained by dilution of latently-infected cells. Our observations were confirmed by measuring the frequency of CD4+ T cells harboring integrated HIV DNA; we observed a significant decreased over time (from 113 to 2253 copy/106 CD4+ T cells at BL (mean of 949) versus 56 to 1581 copy/106 CD4+ T cells at month 26-39 (mean of 533). The protective effect of the CCR5 gene disruption was shown in an independent trial where SB-728-T led to control of viral replication upon cessation of antiretroviral therapy (ART); this correlated with frequencies of CCR5 gene edited TSCM and their TEM progeny. This suggests that the generation of HIV protected memory CD4+ T cells will improve T cell homeostasis and contribute to the development of an HIV cure. These promising results have triggered the development of a new two arm clinical trial currently funded by DAIDS and NIAID. In the current project immune reconstitution and HIV reservoir decay will be monitored using approaches similar to those described above.

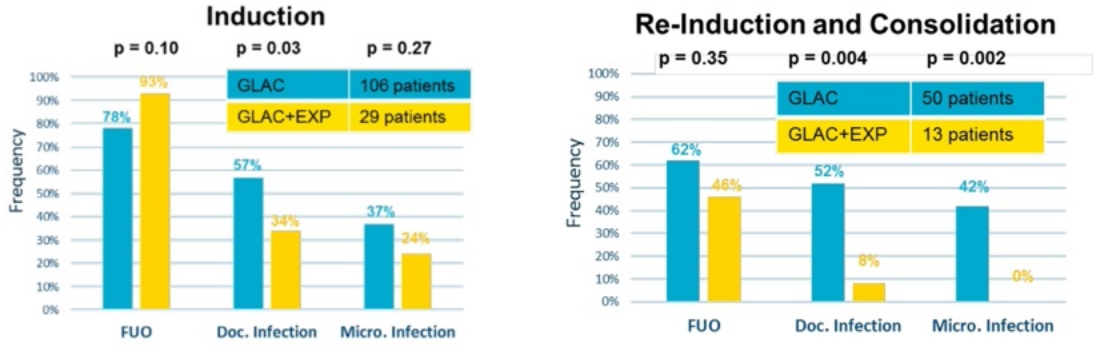
2.3 Clinical Data to Date

Proof of Principle: Preliminary Results of a Phase I UCB alloSCT Trial Using Ex Vivo Expanded Cord Blood Progenitors (FHCRC 2044): The first clinical trial using Notch-mediated ex vivo expanded UCB HSPC was initiated in 2006 in the myeloablative DCBT setting, in which one of the two selected UCB 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 UCB 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) [1].

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. 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 Δ 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".

Based on the promising data using Notch-mediated expanded UCB hematopoietic progenitors in CBT, we developed a cryopreserved universal donor cell product, immediately available to any patient regardless of HLA match, for on-demand use. Using this next-generation universal donor approach, we conducted another Phase I trial to investigate the safety and preliminary efficacy of infusing dilanubicel following intensive chemotherapy for adult patients with acute myeloid leukemia (AML) [40]. This trial enrolled 29 adult AML subjects (19 newly diagnosed and 10 relapsed/refractory) to test the safety of infusing the dilanubicel after induction and consolidation chemotherapy with clofarabine, cytarabine and G-CSF priming (GCLAC). No unexpected toxicity or transfusion-related GVHD was observed during the trial. Although the study was not powered to look at clinical efficacy of the dilanubicel on rates of infection, our treated patients were compared to a current control cohort of 106 patients given GCLAC alone on alternative trials. As shown below in **Figure 3**, patients receiving expanded cells experienced a significant reduction in documented and microbiologically documented infections after induction and consolidation.

Figure 3: Frequency of infections after induction and re-induction/consolidation. Subjects who received chemotherapy + dilanubicel (GCLAC+EXP) were compared to patients who received GCLAC alone on a different protocol.



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 and results were compared to our historical cohort of patients receiving myeloablative CBT. The median age was 21 years (range, 5-45) and weight 59 kg (range, 23-89). Four patients (26%) received only 1 unmanipulated UCB unit along with the expanded graft. All patients engrafted at a median of 19 days (range, 9-31 days), significantly faster than the 25 days (range, 14-45) observed in 50 recipients of two unmanipulated units otherwise treated identically (p=0.01). Importantly, no transplant-related-mortality 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 5.6 years **(Figure 4)**. 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 UCB unit(s). In this pilot study, it appears that infusion of DCC-UCB is associated with less severe acute GVHD (**Figure 3**). The results of this pilot study showed that the use of a cryopreserved non-HLA matched product was not only feasible and safe, but it was also associated with excellent clinical outcomes. Based on these promising results, a prospective multicenter randomized trial (FHCRC Protocol 2603) utilizing this product is underway.

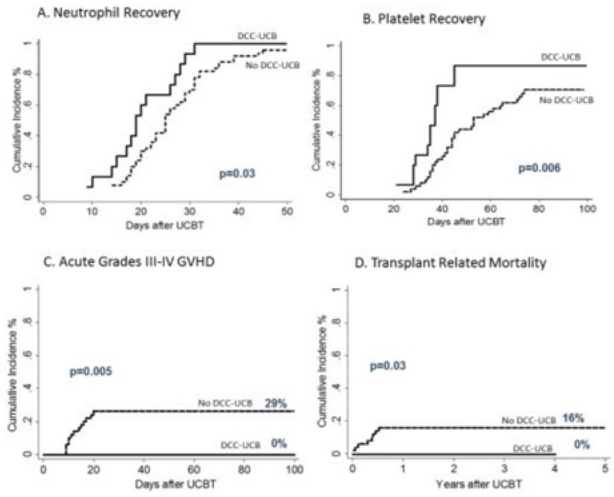


Figure 4: Infusion of DCC-UCB: results in faster hematopoietic recovery (A, B) and reduced severe acute GVHD (C) and Transplant Related Mortality (D).

2.4 Investigational Agent

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, Δ1. Dilanubicel contains no T cells and is used as an off-the-shelf, universal donor product with no requirement for HLA matching. 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 is fully tested and released for clinical use prior to being added to available inventory. Refer to the Investigator's Brochure for product details.

2.5 Regimen Rationale

As discussed above, it is important to note that the expanded cell product contains no mature T cells, and therefore should be able to be infused in a recipient who has undergone a myeloablative preparative regimen. As in the previous trial, we do not anticipate that the expanded unit will be the long-term engrafting unit, due to likely eventual (or immediate) rejection mediated by the non-manipulated unit(s). The primary goal of this study will be to evaluate the safety of infusing an expanded progenitor UCB unit as an additional source of cells capable of rapid but transient myeloid recovery in a conventional myeloablative, non-manipulated CBT.

This clinical trial will be identical in preparative regimen, GVHD prophylaxis, and supportive care as per institutional guidelines to our current Phase II trial (Fred Hutch 9910) for patients with hematologic malignancy who do not have HIV infections. As in Fred Hutch Protocol 2010, stopping rules for infusional toxicity and graft failure will also be in place. The major differences are donor selection for the unexpanded cell product and the use of one unmanipulated unit, allowing for engraftment of a specified unit with CCR5 32 mutation.

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 UCB 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 below for more details.

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 can 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 the expanded product 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).

2.6 Risks/Benefits

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 150 infusions of dilanubicel have been performed in the setting of cord blood transplant and chemotherapy induced neutropenia and treatment is generally well tolerated. 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 randomized protocol (Protocol 2603) has utilized similar cell dose ranges. However, the possibility of grade 3 and grade 4 infusion toxicity exists and as such, monitoring requirements are included in this protocol from infusion through 24 hours.

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 UCB 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. Dilanubicel is used to augment this conventional UCB 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.

Potential Benefits

The use of UCB 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.

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 patients with hematological malignancies and HIV infection in need of alloSCT when dilanubicel is 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 UCB 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). 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 UCB graft.

If we can demonstrate the safety of this approach, the potential impact is tremendous, and provide proof of principle to justify studies of nontoxic regimens for curative therapy of HIV in patients who do not have hematologic malignancy.

3.0 STUDY OBJECTIVES

3.1 Primary Objectives

This multi-institution prospective, single arm feasibility study will evaluate transplantation of an off-the shelf ex vivo expanded progenitor UCB product (dilanubicel) to facilitate engraftment of unrelated cord blood units that have been characterized for the CCR5 32 mutation.

The primary objective is to show that single unit cord blood transplantation characterized with the CCR5 32 mutation augmented with a single dose of dilanubicel is not associated with an excess of graft failure.

3.2 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 year

4. Determine non-relapse mortality at day 100 and 180
5. Assess CCR5Δ32 cord blood stem cell engraftment and its effect on biomarkers of HIV-1 infection, including plasma viral load and replication-competent reservoirs, as well as in gut and other sites (if tissue samples are available).
6. Examine immune homeostasis and reconstitution after transplantation with CCR5Δ32 cord blood stem cells.
7. Assess the changes in HIV-1 induced inflammatory effects (systemic inflammation and activation of innate and adaptive immune cells) and HIV-1 specific immune responses (antibody and T cell responses) pre- and post- CCR5Δ32 cord blood stem cell transplantation in all cohorts.
8. Evaluate time to HIV rebound and viral kinetics following ART cessation

3.3 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. Obtain preliminary data on the incidence of infections/viral reactivation from the start of conditioning to 100 days post-transplant and then at 6 months, 1 year and 2 years post-transplant as possible.

4.0 STUDY DESIGN

4.1 Description of Study

This is a Phase II prospective, open label, multi-site, non-randomized study. Ten patients will be enrolled over the next two years and treated at the following study sites: Fred Hutch (with its Cancer Consortium partners, University of Washington and Seattle Children's Hospital; Filippo Milano, PI), Case Western Reserve University – University Hospitals (Kirsten Boughan, PI), Children's Research Institute/Children's National Medical Center (Blachy Davila-Saldana, PI), and University of California-San Francisco (Timothy Henrich, PI) (**Figure 1**).

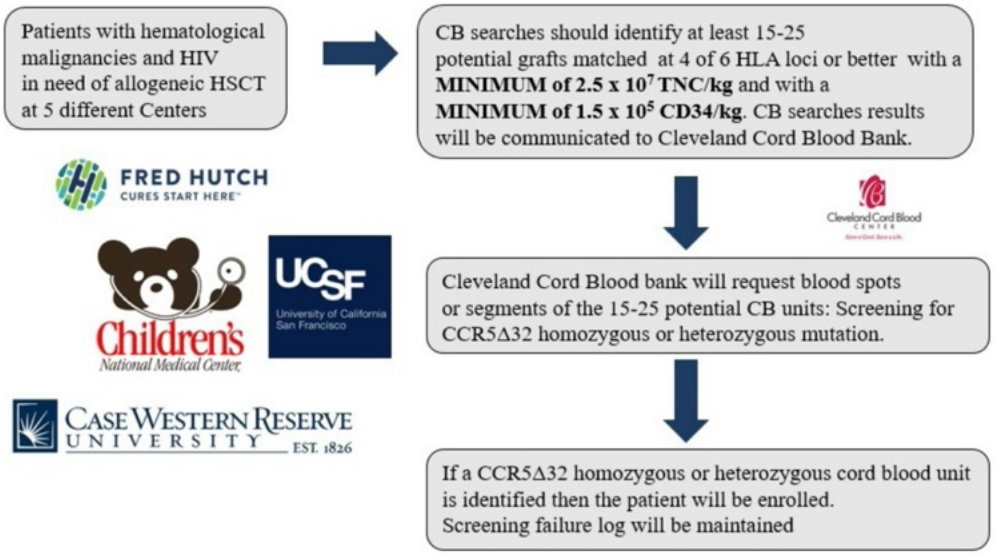


Figure 1: Study enrollment design

Per standard medical practice, preliminary allogeneic unrelated search via National Marrow Donor Program web-based search engine will be conducted for hematology patients infected with HIV who are determined to be eligible for enrollment. Preliminary searches generally identify approximately 16-24 potential UCB grafts based on HLA 4 of 6 match at HLA-A, -B, -DRB1 loci. Blood spots that are routinely maintained by all UCB public banks worldwide will be obtained from UCB banks for UCB grafts identified on this preliminary search to be suitable for CCR5Δ32 allele screening using a PCR assay that allows discrimination whether the genomic DNA are wild type, homozygous, or heterozygous. If a blood spot is not available, alternatively an attached segment can be obtained for PCR testing of an expected 5-8 UCB grafts of the approximately 16-24 identified that would contain sufficient cell dose, with testing performed at Cleveland Cord Blood Center using a PCR method we have developed and validated. If a UCB graft is confirmed CCR5Δ32 homozygous or heterozygous, it will be shipped cryopreserved per standard medical practice to the transplant site, and the dilanubicel Notch-based UCB expanded cell product will be shipped from Fred Hutch to the transplant site, prior to initiating administration of myeloablative conditioning. Samples for correlative studies will be shipped from the transplant center to Fred Hutch that will serve as central repository, and distribution to appropriate laboratories for analyses as outlined in this proposal. Immune monitoring (CWfRU; Sekaly) and assessment of HIV reservoir in blood and tissues (UCSF; Heinrich) will be performed and a bioinformatic approach will allow integration of all data sets to generate biomarkers of successful immune reconstitution and HIV reservoir decay.

4.2 Endpoints

4.2.1 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.

4.2.2 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
5. HIV plasma viral load will be monitored pre transplant and post-transplant by Dr. Heinrich at UCSF.
6. Immune homeostasis and reconstitution will be evaluated by Dr. Sekaly at Emory University.
7. Changes in HIV-1 induced inflammatory immune responses will be evaluated by Dr. Sekaly at Emory University.
8. Assessment of HIV rebound and viral kinetics following ART cessation will be done according to our Infectious disease group.

4.2.3 Exploratory Endpoints

1. In vivo persistence of dilanubicel
2. Duration of initial hospitalization from day 0 post-transplant
3. Overall survival at 1 year
4. Immune reconstitution: assess the kinetics of immune system recovery as measured by T and B cell subsets

4.2.4 Estimated Accrual

Approximately 10 subjects will be enrolled.

5.1 Inclusion Criteria

5.1.1 Age Criteria

≥ 6 months to ≤ 65 years

5.1.2 HIV Criteria

A. Treatment with combination antiretroviral therapy (cART) for at least 1 month before enrollment.
B. Viral load <5000 copies/ml plasma on cART.

5.1.3 Disease Criteria

A. Acute Myeloid Leukemia
   1. High risk CR1, ≥2 cycles to obtain CR, erythroblastic or megakaryocytic leukemia; ≥ CR2.
   2. All patients must be in CR as defined by hematologic recovery and <5% blasts by morphology within the bone marrow and a cellularity of ≥ 15%.
   3. Patients for whom adequate marrow/biopsy specimens cannot be obtained to determine remission status by morphologic assessment, but have fulfilled criteria of remission by flow cytometry, recovery of peripheral blood counts with no circulating blasts, and/or normal cytogenetics (if applicable) may still be eligible. Specimen for morphologic assessment, including possible repeat procedures will be obtained (as possible). These patients must be discussed with the Lead Principal Investigator, Filippo Milano (206-667-5925 or pager, 206-314-1037) prior to enrollment.

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

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

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

E. Other hematologic malignancy such as non-Hodgkin lymphomas. Fred Hutch site: These patients must be presented at Patient Care Conference (PCC) prior to enrollment, given potential competing eligibility on auto-transplant protocols. Participating centers: These patients must be discussed with the Lead Principal Investigator, Filippo Milano (206-667-5925) or pager, 206-314-1037) prior to enrollment.

5.1.4 Organ Function and Performance Status Criteria

A. Performance status score
   1. Karnofsky (≥ 16 years old) ≥ 70%
   2. Lansky (<16 years old) ≥ 50%

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

C. Hepatic Function

Total serum bilirubin must be <3mg/dL and transaminases must be < 3x the upper limit of normal.

D. DLCO corrected >50% normal or for pediatric patients in whom DLCO cannot be measured has adequate pulmonary function

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

5.1.5 Other Criteria

A. Ability to understand and the willingness to sign a written informed consent document (adult subject or parent/legal guardian of minor subject).

5.2 Exclusion Criteria

A. Uncontrolled viral or bacterial infection at the time of study enrollment
B. Active or recent (prior 6 month) invasive fungal infection without ID consult and approval
C. Pregnant or breastfeeding
D. Prior myeloablative transplant within the last 6 months
E. Extensive prior therapy including > 12 months alkylator therapy or > 6 months alkylator therapy with extensive radiation
F. CNS leukemic involvement not clearing with intrathecal chemotherapy. Diagnostic lumbar puncture to be performed as per **section 9.5**.

6.0 CORD BLOOD GRAFT SELECTION

6.1 Unmanipulated Unit

Donor UCB graft selection will be performed as follows:

a) All unmanipulated grafts considered for transplant will be based on cryopreserved total nucleated cell (TNC) dose and HLAA, B, DRB1 matching using intermediate resolution for A and B antigen and high resolution (allele typing) for DRB1. The UCB unit are recommended to have a minimum TNC of 2.5 x 10^7/kg. The minimum recommended CD34/kg cell dose is 1.5 x 10^5/kg, total dose.
b) HLA matching will not be considered for the off-the-shelf product.

Blood spots are routinely maintained by cord blood banks and will be obtained from all potential units that meet the above criteria and will be sent to Cleveland Cord Blood Center for PCR testing in order to identify homozygous or heterozygous CCR5Δ32 UCB grafts. We will also be testing UCB units with lower TNC in order to establish the incidence of homozygous or heterozygous CCR5Δ32 UCB grafts for each patient.

In case of identification of homozygous or heterozygous CCR5Δ32 UCB grafts that don't meet either the minimum recommended TNC of 2.5 x 10^7/kg or the minimum recommended CD34/kg cell dose of 1.5 x 10^5/kg, we will still allow their use for transplant after approval from the site PI and Lead PI (or an additional participating PI, if transplant occurs at Lead PI's site) and notification of the Sponsor. Cord blood units with TNC less than 1 x 10^7/kg will not be considered for transplant in any circumstance.

After engraftment is safely established in the first 3 patients, we will decrease the minimum recommended TNC dose to 2 x 10^7/kg. The minimum recommended CD34/kg cell dose will remain 1.5 x 10^5/kg, total dose.

6.2 Ex vivo Expanded Unit

Each dose of dilanubicel 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. The product will be shipped to the participating site for outside subjects. Refer to the dilanubicel Handling and Administration Guidelines for instructions.

7.0 INFORMED CONSENT OF SUBJECT

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 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.

Fred Hutch patients: Eligible subjects will be identified and registered into the system by the Clinical Coordinators Office (CCO) (Intake Office) and assigned a UPN (Unique Patient Number). The CCO will register the subject on to the protocol through the Data Management Office. Fred Hutch Subjects will be registered by the Fred Hutch/UW Study Coordinator and entered into the Clinical Trials Management System (OnCore). Complete, signed study consent and HIPAA authorization forms are required for registration.

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

Registration Instructions for Participating Centers:

- Contact the Coordinating Center to alert them to the arrival of a new registration
- Complete the Eligibility Checklist
- Fax or send via secure email the eligibility checklist, source documentation, and a copy of the signed consent form
- Coordinating Center is responsible for forwarding valid registrations along with the study consent to the Fred Hutch Data Management.

Fred Hutch Data Management will assign a study number (Multi-Center ID) and register the subject on the study.

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 6 months-45 years old)

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

Day	Preparative Regimen	Supportive Care/Other
-8	Fludarabine 25 mg/m^2 IV over 30 minutes	
-7	Fludarabine 25 mg/m^2 IV over 30 minutes Cyclophosphamide 60 mg/kg IV	
-6	Fludarabine 25 mg/m^2 IV over 30 minutes Cyclophosphamide 60 mg/kg IV	
-5	Rest	
-4	TBI 165 cGy twice daily	
-3	TBI 165 cGy twice daily	Begin CSA (as per **Section 9.2A**)
-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 (See 10.1.5)	Begin MMF (as per **Section 9.2B**)
+1		Begin G-CSF (as per **Section 9.3**)

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
      - **Fred Hutch**: 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 120mg/kg.
   2. Preparation, administration and monitoring will be according to Institutional Guidelines.
      - **Fred Hutch:** 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 Lead 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). TBI will be delivered in accordance with institutional standard of care.

REGIMEN B: Intermediate Intensity regimen (6 months through 65 years old)

Fludarabine total 150 mg/m^2 (30mg/m^2 IV x 5 days, days -6 to -2), Cyclophosphamide total 50 mg/kg IV x 1 day, day - 6), Thiotepa total 10 mg/kg (5 mg/kg/day x 2 days, -5 to -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 4 hours	
-4	Fludarabine 30 mg/m^2 IV over 30-60 minutes Thiotepa 5 mg/kg IV over 4 hours	
-3	Fludarabine 30 mg/m^2 IV over 30-60 minutes	Begin CSA (as per **Section 9.2A**) Begin MMF (as per **Section 9.2B**)
-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 (See 10.1.5)	
+1		Begin G-CSF (as per **Section 9.3**)

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
      - **Fred Hutch**: 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.
      A. **Fred Hutch**: 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). TBI will be delivered in accordance with institutional standard of care.

9.2 Immunosuppressive Therapies

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

A. Cyclosporine (CSA)

Fred Hutch
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.
2. Dose adjustments will be made based on toxicity and CSA levels with a targeted trough level of 200-400ng/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.

Participating Centers

1. Cyclosporine (CSA) beginning on day -3 intravenously in the AM to achieve therapeutic levels per Institutional guidelines.
2. Initial dosing will be per the Institutional guidelines
3. Once the subject can tolerate oral medications, CSA can be converted to an oral form.
4. In case of major CSA toxicity (e.g. CNS neurotoxicity), CSA should be discontinued after consultation with the PI. Subjects may be re-challenged when clinically appropriate and alternative immune suppression should be substituted per Institutional guidelines.
5. Subjects unable to tolerate CSA due to renal impairment should also be considered for an alternative immunosuppressant in addition to mycophenolate mofetil as per Institutional guidelines.
6. Subjects will receive CSA for approximately 6 months in the absence of ongoing GVHD requiring systemic immune suppression. If no history or evidence of GVHD, CSA may be tapered with monitoring for GVHD with the aim to be off immunosuppression by approximately 9 months after transplant. In subjects intolerant of CSA due to renal impairment or other toxicity CSA can be tapered before MMF. This is a reverse taper (according to Institutional guidelines).
7. For subjects with GVHD, CSA may be continued for longer time periods according to standard of care guidelines.
8. If disease progression or persistence occurs, or the subject is considered to be at very high risk of relapse, early taper or cessation of CSA can be considered with close observation for GVHD.

B. Mycophenolate mofetil (MMF)

Fred Hutch

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**. For subjects receiving **Regimen B,** MMF will begin on **day -3.**
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.
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] $\geq 0.5 \times 10^9$/L), whichever day is later, if there is no evidence of acute GVHD and donor CD3 engraftment is at least 50% from 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 Lead PI, Filippo Milano (pager: 206-314-1037).

Participating Centers

1. MMF will begin on **day 0**, starting approximately 4 - 6 hours after infusion of dilanubicel is completed for subjects receiving **Regimen A**. For patients receiving **Regimen B**, MMF will begin on **day -3** intravenously in the AM. Standard dose for adults is 15 mg per kg per dose IV q8 hours
2. Obtain therapeutic trough levels as per Institutional guidelines and adjust per guidelines (max dose 1500 mg q8h) on days +1 and +8.
3. In preparation for discharge, switch to oral route (CellCept or generic mycophenolate mofetil). For oral conversion round to tablet size. If possible, ensure both tablet strengths are given to patient to permit easy taper in clinic. Do not use suspension.
4. No dose adjustments for renal or liver disease are needed routinely unless severe organ dysfunction.

5. If patient is > +28 days and without neutrophil engraftment, consideration can be made to dose reduce dosing after discussion with the PI.
6. If no evidence of GVHD, MMF can be tapered at approximately 60-100 days post-transplant. Taper at 10-20% decrements. The aim to be off the drug by approximately 4-6 months. Earlier tapers can be considered if myelosuppression or high relapse risk with very close monitoring for GVHD. Abrupt reductions or cessation should be avoided due to GVHD risk.
7. Patients who are intolerant of MMF due to myelosuppression may require earlier taper at the treating physician's discretion. Do not abruptly stop the drug unless life-threatening toxicity is suspected.
8. If the patient is intolerant of CSA, MMF taper may be delayed i.e. do a reverse taper where CSA is tapered first (see above).
9. If the patient has acute GVHD requiring systemic therapy, MMF should only be tapered if control of GVHD has been obtained.

9.3 Growth Factor

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

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

9.5 CNS Prophylaxis and Testicular Irradiation

1. All patients will receive a spinal tap during the pre-admission workup following institutional guidelines for diagnostic tap alone or therapy.
2. No dose of intrathecal therapy is to be given less than 72 hours before marrow/stem cell infusion. Beginning on day 30 post-transplant, patients will receive IT Methotrexate as indicated, per institutional guidelines.
3. The CNS prophylaxis regimen may be modified by the attending physician in consultation with the clinical coordinator as clinically indicated (i.e., intrathecal methotrexate can be omitted in patients with a low risk of CNS relapse or in patients who have a high risk of leukoencephalopathy).
4. Testicular irradiation. Male patients who are receiving 1200 or more cGy TBI for ALL, lymphoid blast crisis of CML will receive a prophylactic 400 cGy testicular irradiation boost pre-transplant during the conditioning regimen.

9.6 Antiretroviral Prophylaxis-Highly Active Antiretroviral Therapy (HAART)

1. Definition: Preferably HAART should include reverse transcriptase inhibitors plus either non-nucleoside reverse transcriptase inhibitors or protease inhibitors or both. **Fred Hutch:** Other HAART regimens will be allowed after approval by the Infectious Disease service; **Participating Sites:** Refer to institutional guidelines. Preference will be given to the combination therapy with which the patient has been treated prior to referral to the transplant center.
2. The Infectious Disease (ID) service will be consulted to evaluate each patient and advise management of antiretroviral therapy and infection prophylaxis. Patients will continue to receive effective anti-retroviral therapy throughout treatment. The HAART should include Maraviroc when possible, i.e., when the trophile assay indicates a CCr5-trophic virus and if there are otherwise no contraindications
3. HAART: Patients may be co-enrolled on a separate protocol that specifically dictates the HAART regimen or timing. All other patients will receive HAART medications during conditioning and post-transplant. Oral medications that are vomited within ½ hour of administration should be re-administered, otherwise the dose should not be given until the next scheduled time. It is expected that oral medications may not be tolerated at certain periods after HCT, such as during periods of severe mucositis. Every effort should be made to resume administration of HAART at the earliest possible time, to avoid prolonged time without antiretroviral drugs. If medications in the HAART regimen are thought to be contributing to

organ dysfunction, the Infectious Disease Service should be consulted for recommendations as to alternative combination therapy.

9.7 Criteria for HAART Treatment Discontinuation

This protocol will not involve making decisions about HAART treatment discontinuation. Treatment decisions are the responsibility of the clinical health care providers. Continuation of HAART before, during, and after the transplant is desirable; however, the presence of HAART requires additional considerations. First, the development of resistance to HAART is facilitated by intermittent adherence to HAART. Thus, if a participant is only able to intermittently take HAART due to nausea, vomiting or mucositis, then complete discontinuation of all HAART until oral medications can be tolerated is better than intermittent dosing. Further, because of the variable half-life of the HAARTs, if all are stopped at the same time, those with a long half-life remain in the system resulting in functional monotherapy for a period of time until all drugs are cleared. Therefore, when stopping medication, particularly the non-nucleoside reverse transcriptase inhibitors (NNRTI), it is best to continue the reverse transcriptase inhibitors for 1 week following discontinuation of the NNRTI. Finally, and most importantly, there are numerous drug interactions with the HAARTs, especially the protease inhibitors (PI), with ritonavir being the most difficult agent to manage. Please refer to package inserts for general information about the known and/or more common drug interactions. The transplant clinicians are encouraged to review the participant's specific regimen and the potential drug interactions as soon as a transplant is contemplated.

Any participant on a high dose ritonavir regimen should be changed to an alternate regimen prior to transplant. However, even low dose ritonavir at levels used to boost the other PI will lead to significant drug interactions. Therefore, whenever possible, clinicians should consider changing participants to an alternate regimen as far as possible before the transplant. A raltegravir-based regimen has the fewest interactions, followed by NNRTI, and then PIs other than ritonavir.

The protocol does not manage antiretrovirals but advises that there be an Infectious Disease Consultant on-site assisting in the management of the participant's ARV and that the participant stay on appropriate antiretrovirals if possible throughout the posttransplant period and minimally until donor engraftment is complete and the immune system has recovered for at least one year.

9.8 Supportive Care

1. Herpes Simplex Virus (HSV): Patients should receive prophylaxis for HSV infections as per Institutional guidelines.
2. Antifungal prophylaxis: Patients should receive prophylaxis for fungal infections as per Institutional guidelines.
3. Pneumocystis carinii pneumonitis (PCP): All patients will be given PCP prophylaxis before and after HCT as per Institutional guidelines. In addition, CD4 count must be >200 cells/dl and HIV-1 RNA <50 copies/ml for at least 6 months before PCP prophylaxis can be stopped.
4. Cytomegalovirus (CMV): Patients should receive monitoring and pre-emptive therapy for CMV as per Institutional guidelines. In addition to these guidelines, all patients should be evaluated with CMV plasma PCR pre-transplant.
5. Mycobacterium avium complex (MAC): Macrolide prophylaxis for MAC should be initiated prior to HSCT under guidance of ID service in patients with history of MAC or poorly controlled HIV.
6. Tuberculosis risk should be assessed by PPD on all patients. Those patients with positive PPD should receive pyrazinamide and rifampin for 2 months prior to HSCT.

9.9 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:

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.

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.

10.0 EVALUATION

10.1 Study Evaluation

10.1.2 Pre- and Post-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 the chart below for schedule of study evaluations.

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

A. DNA specimen from unmanipulated cord blood unit for chimerism studies (Submitted to Clinical Immunogenetics Laboratory (CIL)).

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Study Table based on Institutional Standard Practice Transplant guidelines except where noted.	Pre-Conditioning	Days Post-Transplant						Long Term Follow-up
		Day 0	Daily to Engraftment	Weekly post-engraftment to Day 80	28 7 days	80 20 days	180 ±30 days	Year 1 and Year 2
Informed Consent	X							
Medical History, Physical Exam	X	X	X	X	X	X	X	X[1]
Performance status	X					X	X	X[1]
AEs and concomitant meds	X	X	X	X	X	X	X (not AEs)	X (not AEs)
Echo or Muga (cardiac ejection evaluation)	X							
DLCO	X							
CBC w differential	X	X	X	Per Usual Care	X	X	X	X[1]
Blood Chemistry	X	X	X	Per Usual Care	X	X	X	X[1]
Panel Reactive Antibody[3]	X	Once at time of count recovery per investigator discretion						
Viral serology/Hepatitis Battery	X							
Pregnancy Test	X							
CMV Surveillance by PCR	X	As clinically indicated or Per Standard Practice Guidelines for Cord Blood Recipients						PRN
Disease Evaluation, which may include BMA and/ or BMBX	X				X	X		X[1,4]

Cord blood transplant		X						
Expanded cord blood Transplant		X						
Chimerism – Peripheral Blood (CD3 and CD33)					X	X		X[1,4]
Immune Reconstitution/homeostasis (Research Studies)[2]	X				X	X	X	X
Comorbidity index score	X							
GVHD Evaluation				X	X	X	X	X[1]

[1]Every effort will be made to complete the 1 to 2-year evaluations as close to these dates as possible, taking into consideration the patient's circumstances at these time points; [2]Immune monitoring and assessment of HIV reservoir in blood will be drawn with clinical labs:[3]Post transplant PRA (Not Standard Practice) will be charged to the Study budget;[4] At year 2 timepoint there is no disease evaluation or Chimerism completed.

10.1.3 Research samples

A. Research Studies
   a. Immune reconstitution/homeostasis will be done as possible pre-transplant, days 28, 80, 6 months, 1 year and 2 years.
      i. **FHCC** sample requirements: minimum of 80 mL, but not to exceed 120 mL EDTA (lavender top) tube. For pediatric patients, 3 mL/kg may be drawn, not to exceed 50 mL total. Samples will be stored for batch shipment to the appropriate laboratories as specified in protocol **section 4.1**.
      ii. **Participating centers sample requirements:** Samples will be collected at all time points as possible and will be shipped to:
      1100 Fairview Avenue N
      M/S: D2-335
      Attn: Katie Kraskouskas / Hadland Lab
      206-667-7279
      Seattle, WA 98109
      iii. FHCC receives samples Monday through Friday ONLY.
   b. OPTIONAL sample collection post-transplant: Leftover samples will be used to meet one of the secondary objectives: CCR5Δ32 cord blood stem cell engraftment and its effect on biomarkers of HIV-1 infection, including plasma viral load and replication-competent reservoirs, as well as in gut and other sites (if either fluid or tissue samples are available). Newly acquired leftover biopsy samples: When a clinically indicated biopsy is to be performed, we will inform the study participant of our request to collect leftover samples. The study participant must provide informed consent prior to collecting leftover samples for research purposes.
      i. **FHCC sample requirements**: Samples will be stored for batch shipment to the appropriate laboratories as specified in protocol section 4.1.
      ii. **Participating centers sample requirements:** Samples will be collected at all time points as possible and will be shipped to:
      1100 Fairview Avenue N
      M/S: D2-335
      Attn: Katie Kraskouskas / Hadland Lab
      206-667-7279
      Seattle, WA 98109
      iii. FHCC receives samples Monday through Friday ONLY.

10.1.4 Unexpanded cord infusion

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. Fred Hutch: See Standard Practice Guideline, *Infusion – Cryopreserved Hematopoietic Progenitor Cells Marrow, Apheresis, or Cord Blood (HPC, Marrow, HPC, Apheresis, HPC, Cord Blood), or Therapeutic Cells (TC, Apheresis)*.
5. The start and stop time of infusion should be recorded on the infusion record.
6. Fred Hutch: 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 site PI (Filippo Milano, (206) 667-5925, pager (206) 314-1037 immediately).

10.1.5 Infusion of dilanubicel (Investigational Product)

1. The cryopreserved investigational 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 the Receipt and Handling Guidelines for instructions.
2. See protocol specific infusion orders. Procedures for infusion of the cord blood unit will be according to Institutional Guidelines. Infuse product as provided by Cellular Therapy.
3. Infusion time of dilanubicel is ideally 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 window of infusion time is 4 hours to 24 hours following completion of the unmanipulated unit.
4. The subject should be premedicated and hydrated per institutional guidelines. Supplemental oxygen for standby use should be present in the room.
5. Monitoring of immediate toxicities: See **Section 12.3.**

10.1.6 Residual/Recurrent Disease Evaluation

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.

10.1.7 Study Evaluation Windows

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

Evaluation Timepoint Post-Transplant	Window
Day +28	± 7 days
Day +80	± 20 days
Day +180	± 30 days
1 year	*
2 years	*

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

11.0 SUBJECT DISPOSITION

Off-study criteria or full withdrawal:

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

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

F. 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 PIs determine that the event is stable. If a subject is lost to follow-up, information will be recorded and documented in the case report form.

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

12.1 Cyclophosphamide and Other Conditioning Agents

COMMON, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, more than 20 and up to 100 may have:
• Hair loss, skin changes, rash, change in nails • Nausea, vomiting, diarrhea, loss of appetite, pain in belly • Sores in mouth • Infection, especially when white blood cell count is low • Absence of menstrual period which may decrease the ability to have children • Blood in urine

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, from 4 to 20 may have:
• Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require transfusions • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Loss or absence of sperm which may lead to an inability to father children • Stuffy nose • Scarring of the lungs which may cause shortness of breath • Fluid around the heart

RARE, AND SERIOUS In 100 people receiving Cyclophosphamide, 3 or fewer may have:
• Severe skin rash with blisters and peeling which can involve mouth and other parts of the body • Damage to the heart or heart failure which may cause shortness of breath, swelling of ankles, cough or tiredness • A new cancer including cancer of bone marrow (leukemia) caused by chemotherapy • Swelling of the body including the brain which may cause dizziness, confusion

Fludarabine

COMMON, SOME MAY BE SERIOUS In 100 people receiving Fludarabine, more than 20 and up to 100 may have:
• Infection, especially when white blood cell count is low • Vomiting, loss of appetite • Tiredness, fever • Pain

COMMON, SOME MAY BE SERIOUS In 100 people receiving Fludarabine, more than 20 and up to 100 may have:
• Bruising, bleeding • Cough • Increased risk of unusual infections lasting more than 6 months

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Fludarabine, from 4 to 20 may have:
• Anemia, kidney problems which may cause tiredness, bruising, or swelling • Nausea, chills • Feeling of "pins and needles" in arms and legs • Damage to organs (brain, lungs, others) which may cause tiredness, changes in thinking or shortness of breath • Confusion

RARE, AND SERIOUS In 100 people receiving Fludarabine, 3 or fewer may have:
• Kidney damage which may require dialysis

Thiotepa

COMMON, SOME MAY BE SERIOUS In 100 people receiving Thiotepa, more than 20 and up to 100 may have:
• Nausea, vomiting • Pain • Loss of appetite • Tiredness • Hair loss • Rash

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Thiotepa, from 4 to 20 may have:
• Infection, especially when white blood cell count is low • Anemia which may require transfusion • Bruising, bleeding • Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require transfusions • Absence of menstrual period • Loss or absence of sperm • Blurred vision • Blood in urine • Swelling of feet or lower legs

RARE, AND SERIOUS In 100 people receiving Thiotepa, 3 or fewer may have:
• Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Cancer of bone marrow caused by chemotherapy

12.2 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
• Nausea and vomiting • Diarrhea • Cataracts • Sterility • Endocrinopathies • Growth failure • Intestinal cramps • Mucositis

LESS FREQUENT Occurs in 5-20 people out of 100
• Parotitis • Interstitial Pneumonitis • Generalized mild erythema • Veno-occlusive disease

UNCOMMON Occurs in < 5 people out of 100
• Dysphagia • Vertebral deformities • Nephropathy

12.3 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 10.1.5 for infusion guidelines)

1. Immediate toxicities: Immediate toxicities (those occurring either during or within the first 24 hours following the infusion of the expanded cord blood unit) might occur. These symptoms could include fever, chills, fatigue, dyspnea, chest tightness or myalgias; alteration in vital signs such as hypotension, tachycardia, tachypnea, or hypoxemia; and skin changes such as erythema, urticaria, or other rash. Such symptoms will be managed by acetaminophen, diphenhydramine, intravenous fluids and supplemental oxygen. Severe toxicity, including but not limited to, fever (>38.5^{0}C), hypotension (adults: systolic BP <90 mm Hg and ≥ 20 mm Hg below baseline), tachycardia (adults: HR >130), tachypnea (adults: RR >32) or hypoxemia (arterial O2 saturation <90%) will be evaluated and managed by the clinical team. The infusion may be terminated by the team if deemed clinically necessary, followed by supportive medical care. 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.

2. 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 17**). 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.

12.4 Granulocyte Colony Stimulating Factor (GCSF)

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

12.5 Cyclosporine

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

12.6 Mycophenolate Mofetil (MMF)

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

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

B. Adverse Events: MMF may be associated with vomiting and diarrhea, decline in hematocrit and white blood cell count, and infection. In the setting of marrow transplantation, several etiologic factors may contribute to alterations in G.I. and hematologic parameters. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the PI. Dose adjustments are described in **Section 9.2**.

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	

13.1 Adverse Events

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.

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.

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
- 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 require therapeutic intervention or a change in subject management

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

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.

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.
- 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.

5) Adverse Event Reporting Period

All AEs including serious AEs (SAEs), regardless of relationship to dilanubicel or transplant, will be monitored and recorded in study-specific case report forms (CRFs) from the time of signing the informed consent until Day 80 +/- 20 days following transplant. After Day 80 +/- 20 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 at any time, data will be captured in CRFs specific to those events rather than as AEs. Only those AEs considered by the investigator to be related to dilanubicel will be recorded. Death at any point during the study follow-up evaluation period will be reported; however, death caused by disease progression will not be considered an SAE.

13.2 Adverse Event Reporting Requirements

1) IRB Reporting

a. All Research Sites

Each site investigator must report adverse events which in the opinion of the principal investigator are unexpected, related or possibly related to dilanubicel, 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.

Investigators 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.

Investigators 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.

b. Fred Hutch only (Coordinating Center)

In accordance with Fred Hutch institutional policy, AEs from any participating site that affect the risk assessment of the trial as a whole (indicates that the research places research participants or others at a greater risk of harm) and those occurring at Fred Hutch as a participating site which in the opinion of the Fred Hutch principal investigator meet all three of the following criteria will be reported to the Coordinating Center IRB after he or she first becomes aware of the problem.

Expedited reporting criteria:

1. unexpected with regard to what is known about dilanubicel
2. related or possibly related to dilanubicel
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 Coordinating Center IRB.

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

2) Coordinating Center Reporting

Serious adverse events meeting the expedited reporting criteria described above, and severe infusion reactions related to dilanubicel that require termination of the infusion, must be reported by e-mail to the Coordinating Center within 3 working days of the local PI learning of the event.

Adverse events that must be recorded per **Section 13.1** that do not meet expedited reporting criteria will be reported to the Coordinating Center at the regularly scheduled CRF intervals (see **Section 16.0**).

Participating centers must also report (1) any other unanticipated problems or events that place research participants or others at greater risk of harm (e.g., breach of confidentiality) or (2) instances of serious or continuing noncompliance (relative to the IRB-approved protocol, terms of IRB approval, or regulations governing the conduct of human subjects research) to the Fred Hutch Coordinating Center within 3 working days of the local PI learning of the event. If there is uncertainty regarding whether an event requires expedited reporting to the Coordinating Center, the event should be reported.

The following noncompliance events must be reported to the Coordinating Center on an expedited basis whether or not the local PI considers them serious: enrolling a patient who does not meet eligibility criteria, failure to obtain informed consent for research procedures, and administering study drug/product at a dose that is not IRB-approved. A written report must be submitted to the Fred Hutch Coordinating Center with the following information: a description of the event, assessment of whether the event placed a participant or others at increased risk of harm, whether re-consenting has or will occur, whether changes to study documents are required, the plan of action to prevent similar occurrences in the future, and whether the event has been reported to the local IRB on an expedited basis. A case report form is provided for this purpose.

Unanticipated problems that do not increase risk and minor noncompliance may be reported at the regular CRF time points. A link to Fred Hutch IRB policies, including those regarding unanticipated problems and noncompliance, is provided in **Appendix E**.

3) Sponsor Reporting

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

Classification		Reporting Time	Reporting Action	Contact Information
Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team awareness	Email notification to Sponsor's Medical Monitor & ISIOC Administrator	Medical Monitor email: msthakar@fredhutch.org 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 Adverse Events		Per CRF completion guidelines	Record information on appropriate CRFs	NA

*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.

4) FDA Reporting

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 an investigator will be evaluated by the Medical Monitor who will assess the seriousness of the event (see **Section 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 dilanubicel using safety information specified in the dilanubicel 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 CFR 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.

5) NHLBI reporting (Fred Hutch only)

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

14.0 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 7 days 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.

15.0 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, as described in the DSMB Charter. 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. The Fred Hutch will distribute a report from the DSMB to the NHLBI Program Official, the Fred Hutch IRB and to the site PIs of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual.

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 from collaborating centers will be summarized and transmitted as paper case report forms (CRFs), then entered by Coordinating Center staff into the Fred Hutch research database. CRFs must be submitted to the Fred Hutch coordinating center for the following time points: approximately day + 28, +80, and +180 post-transplant and at 1-year and 2-year follow up. CRFs are expected to be submitted as expeditiously as possible after the relevant time points (within 28 days). When possible, primary source documents regarding patient outcomes are collected from the collaborating centers with the CRFs to allow verification of protocol compliance, data accuracy and completeness and full and timely reporting of safety data.

17.0 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	1	2	3
Not Hispanic or Latino	4	3	7
Ethnic Category Total of All Subjects	5	5	10
Racial Categories			
American Indian / Alaska Native	0	0	0
Asian	1	2	3
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	1	2
White	3	2	5
Racial Categories: Total of All Subjects	5	5	10

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

A total of 10 patients will be enrolled in this protocol. The study will be suspended pending further review if, at any time in the study arm, there is sufficient evidence to suggest that the true probability of Grade $\geq$3 infusion toxicity exceeds 10% or the true probability of graft failure exceeds 20%. Sufficient evidence will be taken to be an observed failure rate whose lower one-sided 80% confidence limit exceeds the thresholds listed above (10%

for infusion toxicity, 20% for graft failure). Operationally, these limits will be met if any of the following is observed.

Grade ≥3 Infusion Toxicity: 2/3-8, 3/9
Graft Failure: 2/2-5, 3/6-10

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 ANC engraftment. And while no formal "stopping rule" will be set for this endpoint, should the average time to ANC 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 ANC 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).

18.0 TERMINATION OF STUDY

The IND sponsor, Lead Principal Investigator, and the DSMB may discontinue 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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APPENDIX A PERFORMANCE STATUS SCORE

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.

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

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

ACUTE GVHD ASSESSMENT

Staging by Individual Organ Involvement

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

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

LIVER*: measured by total serum bilirubin

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

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

Score	Adult
1	upper GI (anorexia, nausea, vomiting) with diarrhea of <1000 mL/day
2	1000 – 1499 mL/day diarrhea
3	≥ 1500 mL/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.

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 3x upper limit of normal, AST or ALT 4x upper limit of normal or total serum bilirubin 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures
Serositis	Chest pain or cardiopulmonary comprise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

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

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/ l), 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

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.

APPENDIX D HCT 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 FRED HUTCH IRB POLICIES

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

Adverse Events and Noncompliance are addressed in the following policies:

Policy 1.9: "Noncompliance"

Policy 1.11: "Reporting Obligations for Principal Investigators"

Policy 2.6: "Unanticipated Problems Involving Risks to Subjects or Others"