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Cord Blood ex-vivo MPC Expansion plus Fucosylation to Enhance Homing and Engraftment

Institution Study Number: 2016-0051

Sponsor: MD Anderson Cancer Center

IND Holder: The University of Texas MD Anderson Cancer Center

IND Number: 17378

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Protocol Body

1.0 Objectives

Primary objectives:

a. To evaluate the safety and feasibility of transplantation in which one unit of cord blood is expanded in mesynchymal precursor cell (MPC) co-cultures and the other unit of cord blood is fucosylated with fucosyltransferase (FT)-VI and guanosine diphosphate (GDP) fucose prior to infusion in patients with hematologic malignancies following high-dose therapy
b. To evaluate the time to engraftment using one expanded → and one fucosylated cord blood

Secondary objectives:

a. To evaluate the rate and severity of graft versus host disease
b. To evaluate the rates of infectious complications
c. To evaluate the rates of disease-free and overall survival

2.0 Background

For more than two decades, umbilical cord blood (CB) has been investigated clinically as an alternative source of hematopoietic progenitors for allogeneic transplantation of patients lacking an HLA-matched marrow donor. Fewer T-cells and/or less developed[1] T-cells in CB compared to marrow allows for the possibility that CB grafts will produce less Graft vs. Host Disease (GVHD), the major cause of morbidity and mortality in the[2-4] allogeneic transplant setting. Other potential advantages include the ability to markedly increase the number of allografts available and thus the number of patients who could be transplanted, given the availability and ease of collecting CB from placental veins prior to disposal of the placenta, compared to collecting bone marrow[5] or peripheral blood progenitor cells (PBPCs)[6] from living donors. This source of hematopoietic progenitors has allowed CB banks to target collection of units with human leukocyte antigen (HLA) types such as those of minority African American and Hispanic populations, which are under-represented in the National Marrow Donor Program Registry.[7]

Since the first CB transplant performed by Gluckman's group in 1988, more than 5,000 patients world-wide have received related or unrelated CB transplants for a variety of malignant and non-malignant diseases.[8-23] The progression-free survival rates reported thus far are comparable to results achieved following allogeneic bone marrow transplantation. Moreover, there are many reports of what appears to be less GVHD than that associated with bone marrow transplants, despite the use of CB grafts with substantially more donor-recipient HLA disparity than that tolerated in recipients of marrow[11,12,16,17,23] or PBPC[24-26] allografts. The major disadvantage of CB is the low cell dose, which results in slower time to engraftment and higher rates of engraftment failure when compared to bone marrow transplantation.[27-31] In studies of CB transplantation

published by Kurtzberg[1], Gluckman[1], Rubinstein[1], Rizzieri[2], and Laughlin[2] the median times to an absolute neutrophil count (ANC) of > 0.5 x 10^9/L ranged from 22 to 34 days. Median times to a transfusion-independent platelet count > 20 x 10^9/L varied from 56 to over 100 days, with engraftment failure rates of 12-18%. However, as shown in Table 1, the engraftment failure rate for the adult patients (> 18 years old and/or >45 Kg) in those series was substantially higher, ranging from 10-62%. It is these larger, adult patients, who might benefit the most from the fucosylation of CB progenitor cells as discussed below.

Table 1. Cord Blood Transplants: Engraftment Results

Source	N	Days to ANC (500)	Days to plats. (20K)	Engraft. Failure	Ref.
NYBC-All	**560**	**25**	**71**	**13%**	**[17]**
NYBC-Adults	**97**	**34**	**128**	**46%**	**[17]***
Duke-Peds	**25**	**22**	**82**	**12%**	**[12]**
Duke-Adults	**38**	**26**	**80**	**21%**	**[21]**
Gluckman-All	**143**	**30**	**56**	**18%**	**[16]**
Gluckman-Adults	**23>45 kg**	**NR**	**NR**	**62%**	**[16]***
Laughlin-Adults	**68** **>2.4 TNC/kg** **<2.4 TNC/kg**	**27** **26** **32**	**58**	**10%** **0/5** **5/5**	**[22]**

* Data abstracted for adult patients from reference

Rationale for Expanding Cord Blood Cells *Ex vivo*

Single Umbilical Cord Transplantation

Cord blood recipients receive approximately 1 log fewer mononuclear cells and substantially fewer myeloid progenitors, measured as colony-forming units-granulocyte/macrophage (CFU- GM), than do bone marrow recipients. In most of the cord blood transplant studies referenced above, there appeared to be a threshold effect in the total nucleated cell (TNC) dose of unmanipulated cord blood infused and time to engraftment, i.e., subjects who received doses above the median for a given study appeared to experience superior engraftment as compared with subjects who received TNC doses below the median. In the study by Gluckman,[16] engraftment and survival were superior in subjects who received $\geq$ 3.7 x 10^7 TNC/kg. This large a cell dose is not generally available for subjects weighing more than 45 kg. For adult subjects it appears that recipients of $\geq$ 1.0 x 10^7 TNC/kg had more favorable engraftment than recipients of lower cell doses.[16,22] In another study,[12] a linear correlation between the number of cord blood nucleated cells infused and time to neutrophil engraftment ($p < 0.002$) was reported in the unrelated cord blood transplant setting.

Double Umbilical Cord Transplantation

Brunstein[32] recently analyzed the outcome of 536 subjects who underwent hematopoietic cell transplantation for hematologic malignancies at the Fred Hutchinson Cancer Research Center and the University of Minnesota (USA). In this study, the 128 subjects who underwent double umbilical cord transplantation had a reduced risk of relapse compared to subjects who had the other graft types. In the double unrelated cord blood transplants, the median neutrophil recovery time was 26 days, more than a week longer than the median times for transplants from other donor sources, and median platelet recovery time was at least 4 weeks longer than that following other graft types and often not reached. An important finding of the study was for the 50% of recipients of double unrelated cord blood transplants who recovered neutrophils most rapidly (< 26 days), non-relapse mortality was only 16% compared to 41% in those who recovered later. The difference was primarily due to different rates of infectious complications.

Collectively, these studies suggest giving more cord blood cells may result in faster neutrophil recovery. Expansion of cord blood progenitors *ex vivo* prior to infusion could potentially ameliorate inadequate hematopoietic recovery by generating higher numbers of the cells responsible for short-term reconstitution.

Development of the Mesenchymal Precursor Cells (MPC)-Expanded Cord Blood Product

To date, most studies of MPC-expanded cord blood for clinical use have been performed using CD34+ or CD133+ immunoselection of the cord blood followed by liquid suspension cultures with cytokine supplements.[33] A copper chelator tetraethylenepentamine (TEPA) has also been used to expand the more primitive CD34+ population.[34] A significant limitation of the immunoselection method is the low recovery of CD34+ and CD133+ cells following the positive selection process. In the MD Anderson Cancer Center cord blood expansion study involving CD34+ positive selection, the recovery of CD34+ cells from cord blood units was only 49% (range 4% to 70%).[35] To avoid the unacceptable CD34+ cell losses associated with the positive selection procedure, new approaches were developed.

Ex vivo liquid cord blood expansion removes the primitive hematopoietic progenitors from the hematopoietic microenvironment and relies on the addition of exogenous growth factors to prevent apoptosis and to stimulate proliferation, potentially driving differentiation at the expense of self-renewal. Thus, an alternative approach for *ex vivo* expansion is the co-culture of cord blood cells with MSC components of the hematopoietic microenvironment. Cord blood-MSC co-culture does not require the isolation of CD34+ or CD133+ cells prior to expansion minimizing manipulation and loss of hematopoietic progenitor cells. Starting with cord blood, McNiece[36] demonstrated a 10- to 20-fold increase in TNC, a 7- to 18-fold increase in committed progenitor cells (colony-forming cells), a 2- to 5-fold increase in primitive progenitor cells (high

proliferative potential colony-forming cells), and a 16- to 37-fold increase in CD34+ cells, using an MSC co-culture expansion technique.

As originally developed, it was necessary to generate primary MSCs from every subject or a family member, which severely limited the widespread use of this technology. Many subjects do not have an appropriate family member available who can serve as the MSC donor. Many other subjects, in particular those with acute leukemia, are in fragile remissions prior to transplant and at high risk of relapsing during the 4 weeks needed to generate the MSCs and then perform the co-cultures. An “off-the shelf” source offers major potential advantages over MSCs derived from a family member. First, the cells can be made available for immediate use without the need for lengthy processing or the possibility of contamination during culture. The development of master cell banks from young, healthy volunteers offers a way to bypass disease-related decline in stem cell function providing the optimal source of MSCs for the cord blood co-cultures. Finally, standardization of selection and isolation procedures provides a very reproducible product.

Mesoblast International Sàrl (Mesoblast) has developed mesenchymal precursor cells, an off-the-shelf allogeneic source of mesenchymal cells and is the provider of MPCs to be used for the cord blood unit expansion in this study. Bone marrow cells are harvested from the posterior iliac crest of healthy human donors. The mononuclear cells are immunoselected for stromal enrichment using the STRO-3 monoclonal antibody, subsequently expanded, and cryopreserved to produce a cell bank. The expansion of immunoselected bone marrow mononuclear cells selected for mesenchymal precursors yields a product with defined purity, expression of mesenchymal-precursor-specific markers, and potent biological activity. Further research confirmed the immunological tolerance of the allogeneic MPCs in a variety of nonclinical and clinical allogeneic settings. The MPC product is produced in compliance with Good Manufacturing Practices (GMP).

After process development work demonstrated MPCs were effective in the *ex vivo* expansion of unselected cord blood[37], researchers at MD Anderson Cancer Center treated a cohort of 24 subjects as part of a Phase 2 open-label, noncomparative study (study 2005-0781) using this technology. The results of *ex vivo* expansion of cord blood with MPCs indicate it is feasible to reproducibly generate large numbers of stem and progenitor cells in a cord blood unit.

Name and Description of Investigational Product

The expanded cord blood product is produced by the ex-vivo co-culture of cord blood with MPCs derived from adult bone marrow mononuclear cells. The cord blood unit to be expanded will be thawed and undergo *ex vivo* expansion by co-culture on MPCs. This process requires 14 days, after which the MPC-expanded cord blood is fucosylated with FT-VI and GDP-fucose, followed by a wash and then transported for direct infusion. Four to seven days prior to thawing and washing of the cord blood unit that will undergo expansion, a vial of MPCs is thawed and expanded into culture flasks for 14 days prior

to the fucosylation of the expanded cord unit. In total, the release of the final investigational product, the expanded cord blood unit, may take up to 21 days. Several clinical studies showing safety of both approaches are outlined below.

Findings from Nonclinical and Clinical Studies

Nonclinical Studies

No formal animal safety or efficacy studies were required by the United States (US) Food and Drug Administration (FDA) prior to initiating clinical studies.

Clinical Studies

A Phase 2 study (study 2005-0781) of MPC-expanded cord blood was conducted by researchers at the MD Anderson Cancer Center. Results from this study of 24 subjects with hematologic malignancies indicated the majority of subjects had early recovery of neutrophils and platelets in comparison to historical data from subjects receiving unmanipulated double cord blood transplants, specifically a cohort of similar subjects from the Center for International Blood and Marrow Transplant Research (CIBMTR) database, according to its chief scientific director Mary M. Horowitz, MD, MS (personal communication, 2010).

Ex vivo, MPC-expanded cord blood along with an unmanipulated cord blood unit was infused to 24 subjects who had undergone myeloablative conditioning for the treatment of hematologic malignancies following at least 1 relapse or those with high-risk disease. Each subject received 2 infusions. The first infusion (donor A) used an unmanipulated cord meeting the standard HLA and cell dose requirements (HLA matched 4 of 6 [4/6] or higher and cell dose 1.5 x 10^7 TNC/kg). The second infusion used a cord blood unit (donor B) that also met the standard cell dose and donor HLA matching requirements and additionally was expanded *ex vivo* in co-culture with MPCs.

Patient Demographics and Disposition

The mean subject age was 38.5 years and 58% of subjects were women (Table 2). Eighteen of 24 subjects who received transplants had relapsed or high-risk acute leukemia (4 with acute lymphoblastic leukemia [ALL], 13 with acute myelogenous leukemia [AML], and 1 with myelodysplastic syndrome [MDS]/ALL).

Table 2: Patient Demographics

Variable	Result
Sex, n (%)	
Women	14 (58)
Men	10 (42)
Race, n (%)	
White	14 (58)
Multiracial	7 (29)
African American	1 (4)
Asian	1 (4)
Unknown	1 (4)
Age, years	
n	24
Mean (SD)	38.5 (12.59)
Median	39.45
Min, max	18, 62
Diagnosis, n (%)	
Acute lymphoblastic leukemia (ALL)	4 (17)
Acute myelogenous leukemia (AML)	13 (54)
Chronic lymphocytic leukemia (CLL)	1 (4)
Diffuse, large cell	2 (8)
Hodgkin's disease	1 (4)
Myelodysplastic syndrome (MDS)	2 (8)
MDS/AML	1 (4)
Zubrod scale score at admittance, n (%)	

Mesenchymal Precursor Cells-Expanded Cord Blood Product Characteristics

Subjects received a mean TNC dose of 8.9 $x10^7$ TNC/kg; the mean CD34+ cell dose was 2.1 x 10^6 CD34+/kg. The mean TNC and CD34+ doses in the MPC-expanded cord were 6.4$x10^7$ (standard deviation [SD] 4.0$x10^7$) cells/kg, and 2.0 x 10^6 (SD 2.3 x 10^6) cells/kg, respectively. By comparison, the mean TNC and CD34+ doses in the unmanipulated cord were 2.5 x 10^7 (SD 8.4$x10^6$) cells/kg, and 1.2 x 10^5 (SD 9.5$x10^4$) cells/kg, respectively.

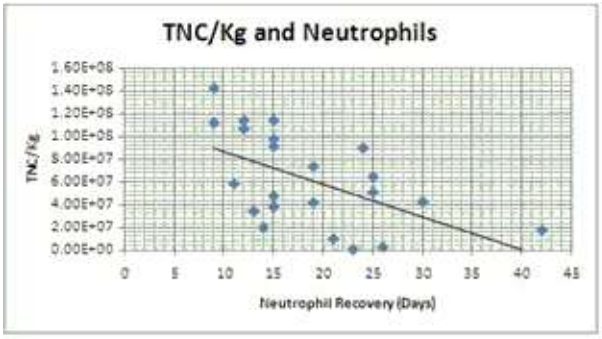
Overall, use of an *ex-vivo* MPC-expanded cord blood cell product resulted in a CD34+ cell dose approximately l log higher than the dose achievable from other cord products in this size population and within the range of CD34+ cells/kg achievable with other progenitor cell types (marrow and mobilized blood cells).

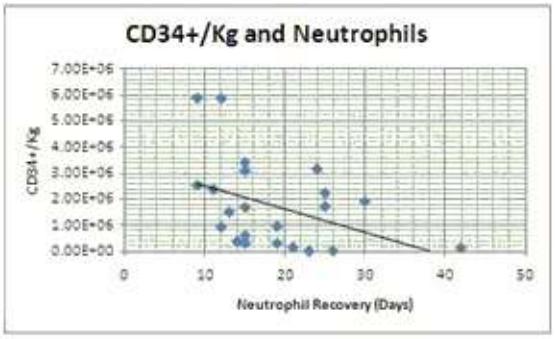
Engraftment Results

The TNC/kg dose of MPC-expanded cord blood strongly correlated with neutrophil recovery (p=0.007) (Figure 1). However, TNC/kg did not correlate with platelet recovery (p=0.792). For CD34+ cells/kg dose, there was a trend toward significance for both neutrophil and platelet recovery. These data strongly support TNC/kg as a relevant

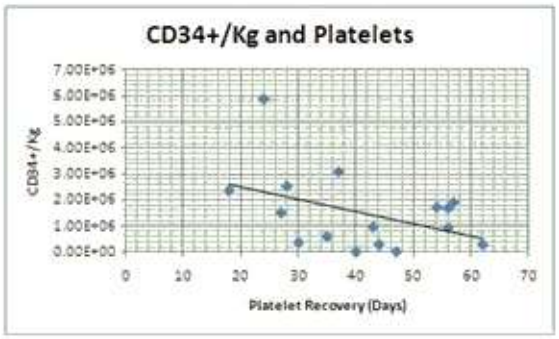
marker for the potency of the MPC-expanded cord blood product for neutrophil recovery. There was also a trend indicating that CD34+ cells/kg dose correlated with both neutrophil and platelet recovery and may therefore also be an indicator of MPC-expanded cord blood product potency.

Figure 1. Correlations of Total Nucleated Cells/kg and CD34+ Cells/kg with Neutrophil and Platelet Recovery





Pearson Correlation Coefficients			
		Neutrophil Recovery	Platelet Recovery
TNC/Kg	correlation	-0.556	-0.072
	p-value	0.007	0.792
	observations	22	16
CD34+/Kg	correlation	-0.413	-0.429
	p-value	0.056	0.097
	observations	22	16



Of the 24 subjects enrolled, complete or partial response was observed in 19 subjects (79%). Of the evaluable subjects, the median time to response was 56.5 days (range 16-116 days); in the 5 subjects who progressed without responding, the median time to progression was 188 days. The range of follow-up was 31 to 426 days. At last follow-up, 16 subjects had died. Causes of death were infection (n=7), GVHD (n=5), progressive disease (n=3), and multi-organ failure (n=1).

Subjects who received *ex vivo* MPC-expanded cord blood cells in addition to an unmanipulated cord blood transplant achieved neutrophil recovery at a median of 15 days (n=23, range 9-42 days) and platelet recovery at a median of 43 days (n=17, range 18-62 days). Notably, 21 of 23 evaluable subjects achieved neutrophil recovery by day 26. Furthermore, platelet recovery occurred in 17 of 21 evaluable subjects by day 100. Overall, 23 subjects had engraftment and there were no engraftment failures (Table 3). One subject was not evaluable for neutrophil engraftment due to death at day 31. Engraftment of neutrophils by 42 days was achieved in all 23 evaluable subjects.

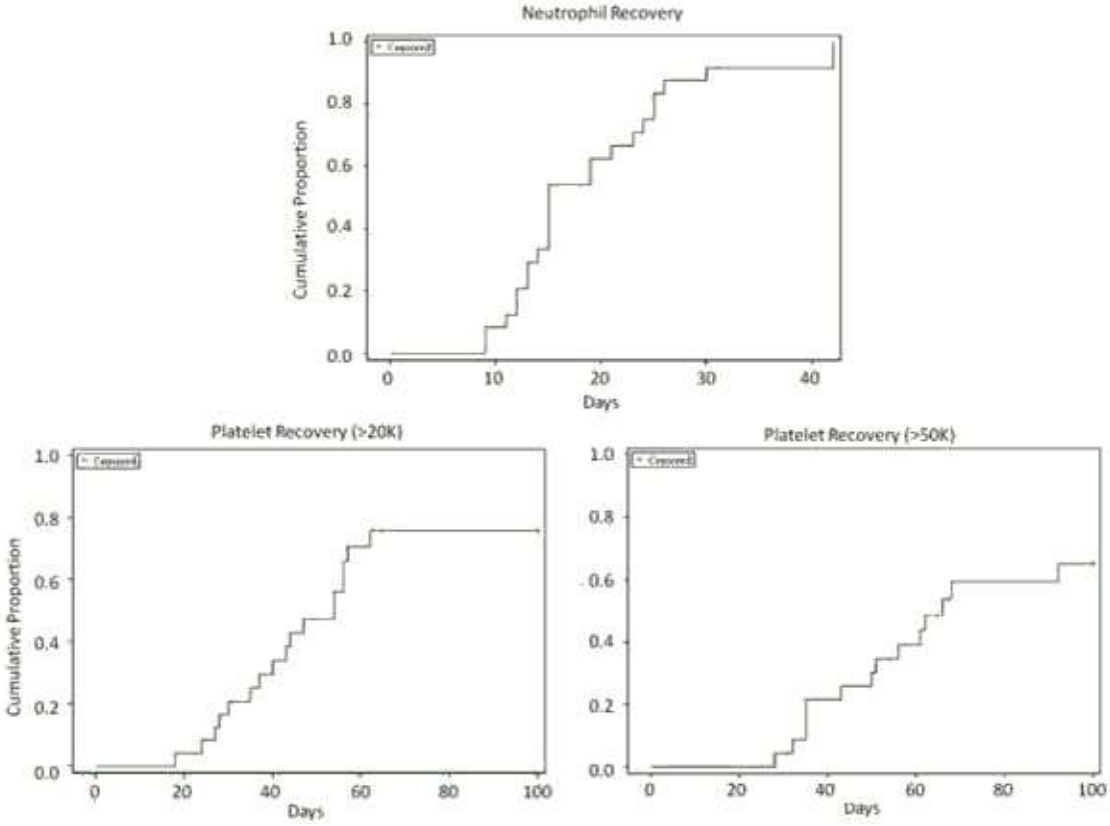
Table 3: Engraftment Results

Parameter	Result	Mean (SD)	Median	Min, max
Number of subjects	**24**	—	—	—
Engraftment, n (%)				—
Yes	23 (96)	—	—	—
Not evaluable	1 (4)	—	—	—
Engraftment failure	0	—	—	—
Time to ANC ≥0.5x10^9/L (days)	23	18 (7.8)	15.0	9, 42
Neutrophil recovery by day 26, n (%)				
Yes	21 (88)	—	—	—
No	2 (8)	—	—	—
Not evaluable	1 (4)	—	—	—
Neutrophil recovery by day 42, n (%)				
Yes	23 (96)	—	—	—
Not evaluable	1 (4)	—	—	—
Platelet recovery to ≥20x10^9/L by day 100, n (%)				
Yes	17 (71)	—	—	—
No	4 (17)	—	—	—
Early death	3 (13)	—	—	—
Time to platelets ≥20x10^9/L (days)	17	42 (13.4)	43.0	18, 62
Platelet recovery to ≥50 000/µL by day 100, n (%)				
Yes	14 (58)	—	—	—
No	6 (25)	—	—	—
Early death	4 (17)	—	—	—
Time to platelets ≥50 000/µL (days)	14	51 (17.9)	50.5	28, 92

ANC=absolute neutrophil count; max=maximum; min=minimum; SD=standard deviation.

Considering the study population as a whole, the median time to neutrophil recovery was 15 days (95% confidence interval [CI] 13.0-23.0) (Figure 2). The median time to platelet recovery to ≥ 20 x 10^9/L was 54 days (95% CI 37-57) and the median time to platelet recovery to ≥ 50 x 10^9/L was 66 days (95% CI 50-no upper boundary). These estimates account for data on subjects who were censored prior to recovery; they differ from the estimates in Table 3, which are based on subjects who recovered.

Figure 2: Cumulative Time to Neutrophil and Platelet Recovery (N=24)



Engraftment duration was monitored by cell counts and chimerism data. All subjects had donor recipient disparity allowing identification of the graft source as donor or recipient (and MPC-expanded or unmanipulated cord blood) by variable number of tandem repeats (VNTR) assay. Both myeloid and T cell chimerism were assessed. Donor T cells were predominantly (≥ 99%) derived from the unmanipulated cord donor in 78% (18 of 23) of the subjects by day 100. Donor myeloid cells were predominantly (≥ 99%) derived from the unmanipulated cord donor in 65% (15 of 23) of the subjects by day 100. Several subjects demonstrated mixed chimerism prior to day 100 but transitioned to unmanipulated cord donor type by the day 100 assessment. These findings demonstrate that the long-term engraftment is predominantly derived from the unmanipulated cord blood, but engraftment rates and times are likely facilitated by the infusion of MPC-expanded cord blood.

Transfusion Results

Platelet and red blood cell (RBC) transfusions were measured to explore hematopoietic recovery times with transfusion requirements and duration of transfusion support. The time to last platelet transfusion (median 54.5 days) correlated with the time to platelet recovery ≥ 50 x 10^9/L (median 50.5 days) more than platelets recovery to ≥ 20 x 10^9/L (median 43 days) (Table 4). Subjects received a median of 146 units of random donor

platelets, which was converted to transfusion episodes by dividing by 6 (size of a platelet pool) to report the number of platelet transfusion episodes (median 24.3) and platelet transfusion episodes per day (until day 100, median 0.5 platelet transfusions per day). Similarly, the RBC transfusion requirements were 16.5 (median) units transfused per subject and the time to last RBC transfusion was 70 days (range 13-165 days). Red blood cell transfusion requirements were not closely associated with times to neutrophil or platelet recovery.

Table 4: Transfusion Outcomes (N=24)

Variable	Mean (SD)	Minimum	Median
Time to last platelet transfusion (days)	65.4 (38.77)	17	54.50
Platelet units transfused	190.4 (121.82)	31	146.0
Units transfused per day	3.2 (1.39)	0.76	3.2
Transfusion episodes	31.7 (20.30)	5.2	24.3
Transfusion episodes per day	0.5 (0.23)	0.1	0.5
Time to last RBC transfusion (days)	71.0 (35.33)	13	70.0
RBC units transfused	19.9 (12.81)	2	16.5

RBC=red blood cell; SD=standard deviation.

Adverse Events

Toxicity and adverse events were expected due to myelosuppression, chemotherapy intolerance, and underlying disease. Although many adverse events were deemed related to the study treatment (transplant), no adverse events or toxicities were considered likely or possibly due to the investigational agent, i.e., *ex vivo* MPC-expanded cord blood cell product or infusion (Table 5). The most common grade 3/4 events post-transplant were dysphagia, elevated bilirubin, infection, hemoptysis, hyperglycemia, somnolence, reduced ejection fraction, and urinary creatinine. Seven subjects had post-transplant events of infection and died.

Table 5: All Adverse Events (Pre- and Post-Transplant)

Parameter	Number (%) of patients
Patients with Adverse Events	**24 (100)**
Adverse events possibly related to study treatment	24 (100)
Maximum grade (CTCAE version 3.0) of adverse events	
Grade 2	2 (8)
Grade 3	14 (58)
Grade 4	6 (25)
Grade 5	2 (8)

CTCAE=Common Terminology Criteria for Adverse Events.

Acute GVHD developed in 16 (67%) subjects. Of these, 3 developed grade 3 or higher GVHD. The time to GVHD onset for grade 3 or higher was 58 days (range 27-118

days). There were no cases of grade 4 GVHD. Chronic GVHD occurred in 10 (53%) of 19 evaluable subjects. Acute and chronic GVHD were the primary cause of death in 1 and 4 subjects, respectively.

The frequencies and descriptions of toxicities related to neutropenia were reported from day 0 to end of follow-up (Table 6). All 23 evaluable subjects had at least 1 diagnosed infection. The time to first infection was 22 days (range 0-97 days). The median times to onset of fever or fever and neutropenia (in the absence of diagnosed infection) were 10 days each (ranges 8-10 and 3-17 days, respectively). Fever with neutropenia was experienced by 11 (46%) of 24 subjects; however, unexplained fever in the absence of diagnosed infection and/or neutropenia was uncommon. Fever in the absence of diagnosed infection occurred more often before day 10.

Table 6: Neutropenia-Related Toxicities Post-Transplant

Parameter	Result	Mean (SD)	Median	Min, max
Patients with toxicity, n (%)				
Viral infection	17 (71)	—	—	—
Bacterial infection	14 (58)	—	—	—
Hemorrhagic cystitis	12 (50)	—	—	—
Febrile neutropenia	11 (46)	—	—	—
Fungal infection	11 (46)	—	—	—
Fever	3 (13)	—	—	—
Pneumonitis	3 (13)	—	—	—
Pulmonary hemorrhage	3 (13)	—	—	—
Patients with infection, n (%)				
Yes	23 (96)	—	—	—
No	1 (4)	—	—	—
Time to first infection (days)	23	24.3 (18.63)	22.0	0, 97
Patients with fever				
Yes	3 (13)	—	—	—
No	21 (88)	—	—	—
Time to first fever (days)	3	9.3 (1.16)	10.0	8, 10
Patients with febrile neutropenia				
Yes	11 (46)	—	—	—
No	13 (54)	—	—	—
Time to first neutropenia (days)	**11**	**8.7 (4.45)**	**10.0**	**3, 17**

Max=maximum; min=minimum; SD=standard deviation

Survival Outcomes

The median survival time for the 24 subjects at both 1 year and overall was 217 days (95% CI 119-272), indicating that 1-year survival is predictive of overall survival in this 24-subject study. At 6 months the median survival time was not reached.

Summary of MD Anderson Cancer Center Study

An analysis of the 24 subjects in the MD Anderson Phase 2 study found the median time to neutrophil recovery was 15 days (95% CI 14-24 days) with 88% (21 of 24) of subjects recovering within 26 days. The median time to platelet recovery was 54 days (95% CI 40-57 days) with 67% (16 of 24) of subjects recovering by day 60.
The noted improvement in neutrophil and platelet recovering is compelling evidence that the graft content of TNC and CD34+ cells correlates with product potency and engraftment kinetics. The marked improvement in platelet recovery indicates an engraftment improvement that has not been consistently seen in cord blood transplantation. Notably, the expanded MPC cord is an engraftment facilitator and does not specifically engraft, as demonstrated by the absence of MPC-expanded cord donor cell types (at day 100) in chimerism studies. The results of the study can be put into context by comparison with historical registry data.

The Center for International Blood and Marrow Transplant Research (CIBMTR) also identified a matched cohort of subjects receiving double unrelated cord blood transplants (N=80) who underwent similar myeloablative conditioning regimens as used in the MD Anderson study. They performed a number of analyses comparing the probabilities of neutrophil and platelet recovery. The analysis showed only 53% (42 of 80) of subjects receiving 2 unmanipulated cord blood units recovered neutrophils within 26 days ($p<0.0001$). Similarly, for platelets, only 30% (24 of 79) of the CIBMTR matched cohorts recovered platelets by day 60 (p-value=0.0008). In addition, the median time to neutrophil recovery was 25 days (95% CI 22-28 days) with 53% (42 of 80) of subjects recovering by day 26. The median time to platelet recovery was 77 days (95% CI 65-over 100 days) with only 31% (24 of 79) of subjects recovering by day 60.

In a separately conducted analysis of subjects by CIBMTR, survival at 6 months was improved for the engraftment composite success group (defined as neutrophil recovery by day 42 and platelet recovery by day 60) (n=24 of 80 subjects) compared to others (81% [95% CI 59%-96%] versus 13% [95% CI 0-42%]), and improved survival at 6 months was predictive of improved survival at 1 year.

Rationale for Fucosylating Cord Blood

A critical part of engraftment is the recruitment of primitive hematopoietic progenitor cells (HPC) to the bone marrow (BM). This process is governed by a cascade of molecular interactions between members of the selectin, integrin and CD44 superfamilies of adhesion molecules expressed by HPC and their receptors expressed by the hematopoietic microvasculature.[38-45] The initial step associated with homing is the rolling of HPC on the vascular endothelium of the hematopoietic microenvironment,[38-48] an interaction mediated by P- and E-selectins constitutively expressed by the BM endothelium and counter-receptors expressed by the HPC.[49] While the delay in engraftment and elevated risk of graft failure observed for CB recipients are thought to be due, at least in part, to low total nucleated cell (TNC)[17,50] and CD34+ cell[51] dose, there

is evidence that CB CD34+ cells may also have a defect in homing to the bone marrow.[52,53] *In vitro* (rolling) and *in vivo* (homing) evidence suggest that CB CD34+ cells interact poorly with selectins when compared to CD34+ cells from PB or marrow. Appropriate carbohydrate modification (fucosylation) of selectin ligands appears to be critical for the rolling of primitive hematopoietic cells on P- and E-selectins expressed by the hematopoietic microvasculature.[47,52,53] An *in vitro* correlation has been demonstrated between the level of CB CD34+ fucosylation and binding to P- and E-selectins.52 More heavily fucosylated CD34+ cells exhibit a greater affinity for P- and E-selectins than less-fucosylated CD34+ cells. The correlation between the level of fucosylation of engrafting hematopoietic cells and their interaction with selectins expressed by the hematopoietic microvasculature highlights an important role for fucosylation in the homing process.

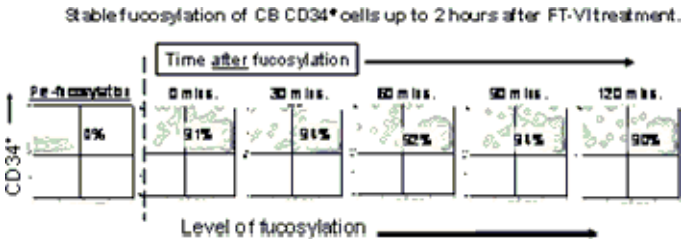
Levels of fucosylation can be markedly increased by *ex vivo* treatment of HPC with a 1-3 fucosyltransferase (FT)-VI.[52,53] Increased levels of fucosylation have been shown to augment the level of P- and E-selectin binding of CD34+ cells as demonstrated by increased levels of rolling on surfaces coated with P- or E-selectin *in vitro* under flow conditions[52], and vascular rolling, through an intravital imaging technique.[53] However the ultimate impact of such *ex vivo* fucosylation on hematopoietic engraftment remains to be documented which is the subject of this clinical trial.

Cord Blood Fucosylation - Results

As discussed above cord blood progenitor cells appear to have a deficit in homing and engraftment. CB hematopoietic progenitor cells have low levels of surface fucosylation. Thus for our recently published phase II study,[54] we postulated that by increasing the level of surface fucosylation might improve interactions with selectins expressed by the microvasculature of the hematopoietic microenvironment, potentially improving homing and engraftment. We used the NOD-SCID IL-2Rgnull (NSG) mouse model[33] to explore the impact of fucosylation on the rate and magnitude of engraftment with the goal of improving engraftment following CB transplantation in the clinic.

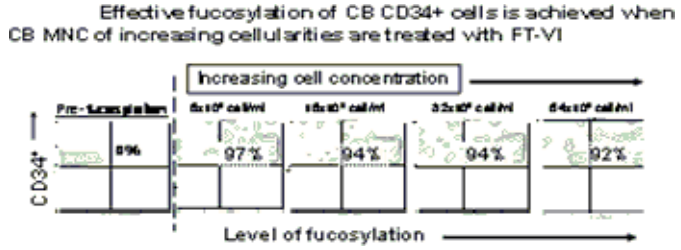
Development of fucosyltransferase (FT)-VI for clinical use. The laboratory conditions for use of FT-VI included incubation of CD34+ cells at 37°C, for 30 minutes with 10 mM manganese in HBSS containing 1% HSA. To refine the procedure for clinical use, we performed flow cytometry with HECA-452 antibody (BD Bioscience) to sialyl Lewis X (sLex)/cutaneous lymphocyte antigen (CLA), a fucosylated selectin ligand, to measure the levels of cell surface fucosylation.[4;83;86] Optimal conditions for the fucosylation of selected CB CD34+ cells required at least 30 minutes of incubation with FT-VI enzyme at *160 mU/ml, at room temperature in HBSS containing 1% HSA and 1 mM GDP ·-fucose and 1 mM MnCl2. (*1 mU FT-VI is defined by the manufacturer as the amount of FT-VI that transfers 1 nMol of GDP-Fuc to Gal1-•-4 GlcNAc-•-PNP in 1 hour.) Fucosylated CB MNC remained stably fucosylated for up to 2 hours as assessed by HECA-452 staining (Figure 3).

Figure 3.



Fucosylation of CB MNC. Since CB CD34+ cell selection is time-consuming, expensive, and results in significant CD34+ cell losses, experiments were performed to determine whether fucosylation of unmanipulated CB mononuclear cells (MNC) would be feasible. Near maximal fucosylation of the CD34+ cells in the CB MNC preparation was achieved over a significant range of CB MNC cellularities (from 8-64x106 cell/ml) (**Figure 4**) suggesting that fucosylation of CD34+ cells in an MNC preparation containing 108 cells/ml is feasible without a reduction in enzyme efficacy.

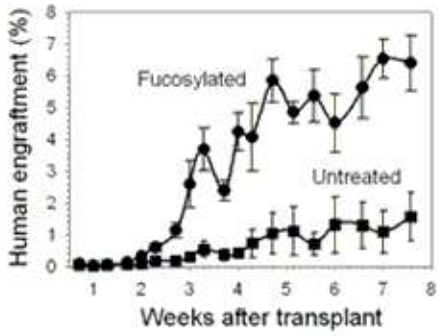
Figure 4.



Improved engraftment after fucosylation of minimally manipulated CB cells. To exploit the *in vitro* conditions in animal studies, we treated CB CD34+ and CB MNC with FT-VI, washed the cells after the fucosylation procedure to remove residual Mn2+ and infused them into sublethally irradiated (270 cGy), NSG mice (5 mice/group): (**A**) CB CD34+ ± FT-VI. Each mouse received ~4.5x104 CB CD34+ cells ± FT-VI treatment cells and was monitored longitudinally via retro-orbital bleeding for the presence of human cells in the peripheral blood by flow cytometry using the human CD45+ antibody. Human engraftment in mice receiving CB CD34+ cells treated with FT-VI was detected more rapidly (earlier) and at a significantly higher level (~4-fold) than in recipients of untreated CD34+ CB cells (**Figure 5A**).

(**B**) CB MNC ± FT-VI. Since the processes associated with the selection of CB CD34+ cells result in significant cell losses and since CB recipients receive unmanipulated (non-selected products) at transplant, the efficacy of fucosylation on engraftment following unmanipulated CB MNC ± FT-VI treatment was investigated. Sublethally-irradiated NSG mice received 6x106 MNC containing ~2.5x104 CB CD34+ cells (5 mice/group). Consistent with results observed when selected CD34+ cells were fucosylated using FT-VI, human engraftment in mice receiving MNC treated with FT-VI was detected more rapidly (earlier) and at a significantly higher(>3-fold) level than in

recipients of untreated CB MNC160 (**Figure 5B**).

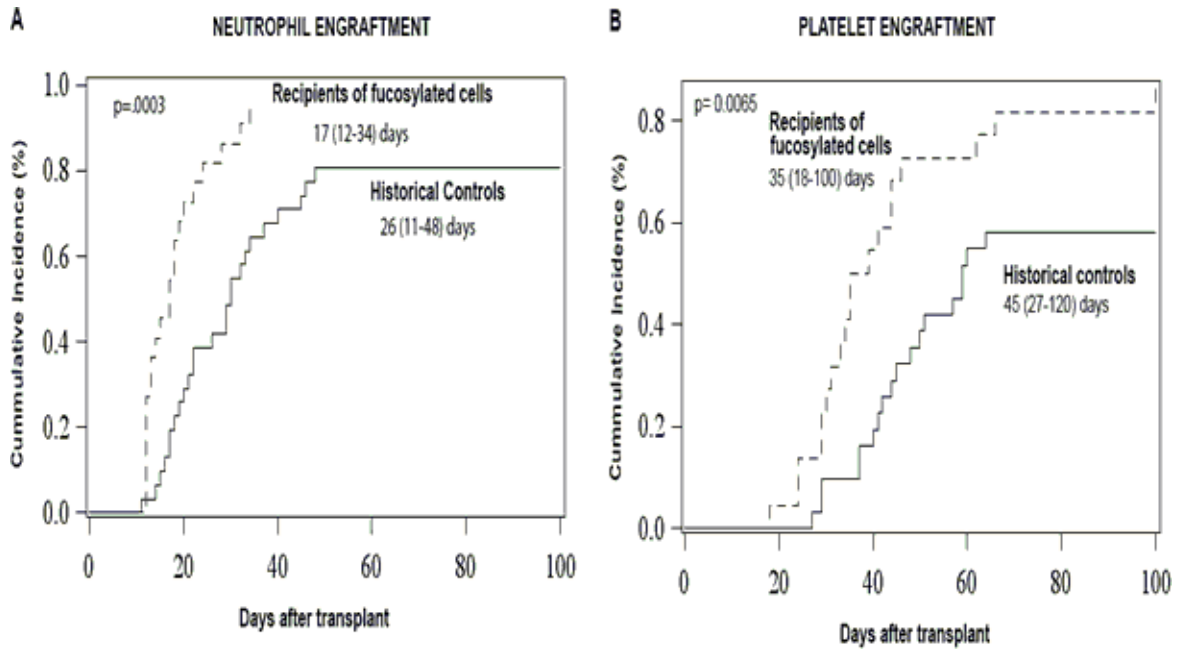


Engraftment measured by the presence of human CD45⁺ cells in the peripheral blood of sublethally-irradiated NSG mice (5 mice/gp) transplnated with with fucosylated or untreated CB CD34⁺ cells.

These data suggest that fucosylation of minimally manipulated CB cells is an effective strategy to improve the speed and magnitude of engraftment. The persistence of engraftment over the longer term (>8 weeks, data not shown) suggests that fucosylation does not negatively impact the long-term repopulating capacity of the graft.

In our Phase II study we found that the median time to neutrophil and platelet engraftment after 30 minutes of fucosylation prior to infusion with one CB unit was 17 days (range 12-34 days) and 33 days (range 18-100 days).54 (Figure 6)

Figure 6 Neturophil and Platelet Engraftment



MSC expanded cord blood has higher endogenous fucosylation than unmanipulated CB. These cells then can be fucosylated to maximally express CLA/sLEX. (Figure 7)

Figure 7. Fucosylation status of Unmanipulated, Expanded and FT-VI Treated CB cells, MSC-expanded CB has higher endogenous fucosylation than unmanipulated CB can then be fucosylated to maximally express CLA/sLex.

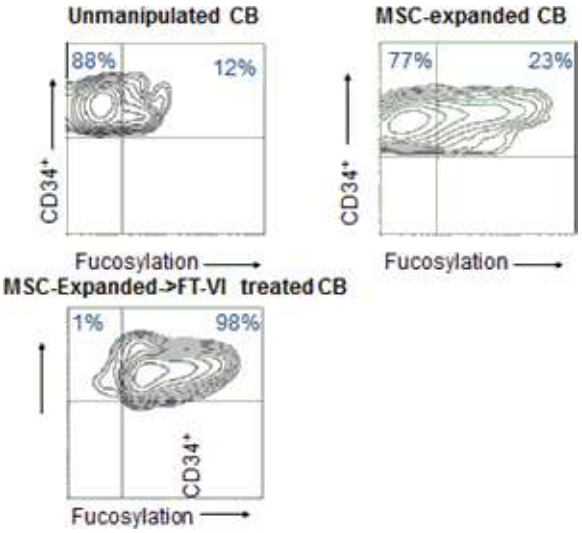
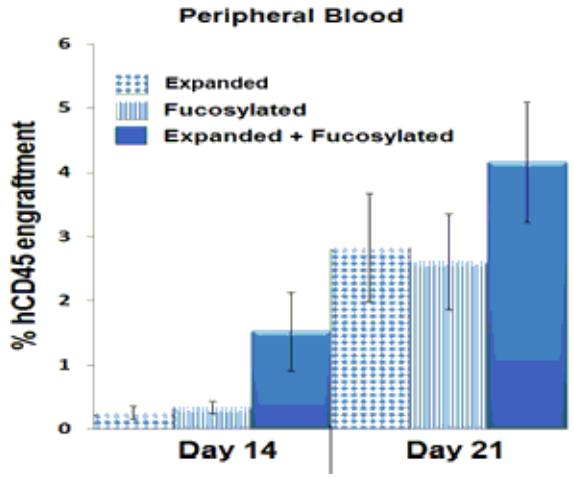


Figure 8 demonstrates in NSG mice, engraftment at Day 7 with Expansion + Fucosylation may be possible.



Double Cord Blood Transplants

In an attempt to increase the number of CB cells infused following high-dose or nonmyeloablative therapy, investigators have combined two units of differing HLA types and infused them as allogeneic hematopoietic support. Barker et al.reported results of patients transplanted with two unmanipulated CB units.68 Eight adults, with a median age of 47 years (range 22-56) and median weight of 81kg (range 63-109), were enrolled on the study. Of 7 patients alive without relapse at day +28, all had 97-100% donor chimerism on day +21 marrow biopsy. ANC recovery of 0.5 x 10^9/L occurred at a median of 25 days (range 21-28). The authors concluded that their data support the principle that transplantation of two immunologically distinct CB units is safe in terms of crossed immunological rejection. Graft failure was not observed, but the majority of patients did engraft with only a single CB unit. In the current trial we will use two CB units, one of which will be co-cultured with MPCs for expansion then fucosylated in attempt to reduce the time to engraftment in CB transplant recipients below the 20-30 days typically reported in this setting.

3.0 Background Drug Information

1. Antithymocyte globulin (ATG)

THERAPEUTIC CLASSIFICATION: Immunosuppressant.

MECHANISM OF ACTION: ATG is a rabbit anti-thymocyte globulin. It is a purified, sterile, primarily monomeric IgG fraction of hyperimmune serum of selective immunosuppressant. It is believed to act by modifying the number and function of lymphocytes.

PHARMACEUTICAL DATA: ATG is diluted in 0.9% NaCl. ATG is given intravenously as a 4-hour infusion into a central vein. It is a colorless to slightly opalescent protein solution. ATG should not be diluted in dextrose or low salt fluids, as this may cause

precipitation. Infusion with acidic solution may cause physical instabilities. Although no further incompatibilities or drug interactions are known, it is recommended to administer ATG alone.

STORAGE AND STABILITY: Commercially available. Solution for infusion contains 1 mg of ATG/2 ml. It should be stored in a refrigerator at 2°C to 8°C (do not freeze). Diluted ATG is stable for 24 hours.

KNOWN SIDE EFFECTS: ATG may cause cytopenia of any cell line, chills, fever, or edema. A serum-sickness like syndrome may also develop. Adverse reactions including rash, pruritus, urticaria, wheal and flare reactions, bronchospasm bleeding and anaphylactic shock have been reported. Resuscitation equipment should be available during administration. After an anaphylactic reaction, infusion should not be resumed.

2. Busulfan

Therapeutic Classification: Antineoplastic Alkylating agent.

Pharmaceutical data: Busulfan injection is a sterile, pyrogen-free solution provided in a mixture of dimethylacetamide (DMA) and polyethyleneglycol 400 (PEG400). It is supplied in 10 ml single use ampoules at a concentration of six (6) mg busulfan per ml. Each ampoule contains 60 mg of busulfan in 3.3 ml of DMA and 6.7 ml of PEG400. When diluted in normal saline or D5W to a concentration of 0.5 mg/ml, the resulting solution must be administered within eight

(8) hours of preparation including the three (3) hour infusion of the drug.

Stability and storage: Ampoules should be stored refrigerated at 2-8°C (35-46°F). Stable at 4°C for at least twelve (12) months. Additional stability studies are in progress. DO NOT use beyond the expiration date. DO NOT use if the solution is cloudy or if particulates are present.

Solution Preparation: Prepare the busulfan solution as follows (the patient will receive their busulfan “test” dose at 32 mg/m2 either as an outpatient prior to admission or as an inpatient on Day -9. Busulfan pharmacokinetics will be performed with the test dose and the day -7 dose and doses for day -7 to day -4 are subsequently adjusted to target an AUC of 4,000 microMol.min-1 then mixed into normal saline to a final concentration of 0.5 mg/mL.

In each bag 6.0 mg busulfan (1.0 ml at 6 mg/ml and 11 ml saline) should be added to compensate for drug lost in the tubing with each infusion (approximately 12 ml at 0.5 mg/ml is lost in the tubing when using the controlled rate infusion pump).

Route of Administration: It is to be noted, that a sufficient amount of diluted busulfan should be added to compensate for the amount needed to prime the IV tubing; when hanging the infusate, the tubing should be primed with the busulfan solution and connected as close to the patient as possible. After completed infusion, the tubing with remaining busulfan (approximately 12 mL) should be disconnected and

discarded. All busulfan infusions should be performed by programmable pump.

The test dose of busulfan is given over 45 minutes and the high-dose busulfan will be given by slow intravenous infusion over three (3) hours into a central venous catheter.

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

An infusion pump will be used with the busulfan solutions as prepared above. A new infusion set must be used for administration of each dose. Prior to and following each infusion, flush the catheter line with normal saline or (approximately 5 ml). Start the three-hour infusion at the calculated flow rate. DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

If a delay in administration occurs after the infusion solution is prepared, the properly identified container should be kept at room temperature (20-25°C), but administration must be completed within eight (8) hours of preparation including the three (3) hour drug infusion.

Adverse Events: Dose limiting toxicity is expected to be hematological when used without stem cell support. Other toxicities seen frequently following high-dose busulfan in preparative regimens for bone marrow transplantation include: VOD, nausea, vomiting, pulmonary fibrosis, seizures, rash, and an Addison's-like syndrome.

Mechanism of action: Interferes with DNA replication and transcription of RNA through DNA alkylation, and ultimately results in the disruption of nucleic acid function.

Animal Tumor Data: Busulfan has been shown to be active against a variety of animal neoplasm in vivo, including mouse sarcoma 180 and Ehrlich's mouse ascites tumor.

Human Pharmacology: Limited pharmacology data are available for the parenteral formulation to be used in this study and is detailed in the evaluation of IV Bu in a Phase II Trial using IV Bu at 0.8 mg/kg BW given over 2 hr every 6 hr for a total of 16 doses (Andersson et al, 2002) and when administered once daily for 4 days at a dose of 130 mg/m2 in combination with Flu (Madden et al, AHS 2003, de Lima et al, BLOOD 2004). The pharmacokinetic data suggests that the plasma decay of the formulation fits an open one-compartment model with linear pharmacokinetics in the dose range of 12 mg-130 mg/m2. Based on studies of oral Bu, the drug is reported to be extensively metabolized; twelve (12) metabolites have been isolated, but most have not been identified. The drug is slowly excreted in the urine, chiefly as methanesulfonic acid. Ten to fifty percent (10-50%) of a dose is excreted as metabolites within twenty-four (24) hours (Nadkarni 1959).

3. Cyclophosphamide

Therapeutic classification: Antineoplastic alkylating agent

Mechanism of action: Interferes with deoxyribonucleic acid (DNA) replication and transcription of ribonucleic acid (RNA) and ultimately results in the disruption of nucleic acid function.

Pharmaceutical data: Lyophilized cyclophosphamide for injection contains 75 mg of mannitol per 100 mg of cyclophosphamide (anhydrous) and is supplied in vials for single-dose use. Storage and stability: Storage at or below 77ºF (25ºC) is recommended; this product will withstand brief exposure to temperatures up to 86ºF (30ºC) but should be protected from temperatures above 86ºF. Reconstituted lyophilized cyclophosphamide for injection is chemically and physically stable for 24 hours at room temperature or for 6 days in the refrigerator; it does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions.

Known side effects: Dose limiting toxicity is hematologic. Nausea and vomiting is common. Anorexia and, less frequently, abdominal discomfort or pain and diarrhea may occur. There are isolated reports of hemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy. Azotemia has been reported. Alopecia and hypersensitivity reactions when high doses are utilized. Hemorrhagic cystitis may develop in patients treated with cyclophosphamide. Rarely, this condition can be severe and even fatal. Acute cardiac toxicity has been reported

with doses as low as 2.4 g/m2 to as high as 26 g/m2, usually as a portion of an intensive antineoplastic multi-drug regimen or in conjunction with transplantation procedures. Interstitial pulmonary fibrosis has been reported in patients receiving high doses of cyclophosphamide over a prolonged period.

Note: It is recommended that administration of azoles should be avoided before and during infusion of cyclophosphamide.

4. Clofarabine

Therapeutic Classification: Antimetabolite; Purine analog

Mechanism of Action: Clofarabine, a purine (deoxyadenosine) nucleoside analog, is metabolized to clofarabine 5'-triphosphate. Clofarabine 5'-triphosphate decreases cell replication and repair as well as causing cell death.

Solution Preparation: Clofarabine is formulated as a concentration of 1mg/mL in United States Pharamacopeia (USP) sodium chloride (9mg/mL), and USP water for injection (qs to 1mL). Clofarabine is supplied in 2 vial sizes: a 10mL flint vial and 20mL flint vial. The 10 mL flint vials contain 5mL (5mg) of solution and the 20mL flint vials contain 20mL (20mg). For both vial types, the pH range of the solution is 4.0 to 7.0. The solution is clear with color ranging from colorless to yellow and is free from visible particulate matter.

Clofarabine should be filtered through a sterile 0.2 micron syringe filter and then diluted with 5% Dextrose Injection, USP, or 0.9% Sodium Chloride Injection, USP, prior to intravenous (IV) infusion to a final concentration of 0.4 mg/mL. Use within 24 hours of preparation.

Clofarabine Formulation and Stability:

Clofarabine vials containing undiluted clofarabine for injection should be stored at 25°C or 77°F with temperature excursion permitted to 15 to 30°C or 59 to 86°F. Ongoing shelf-life stability indicate that clofarabine is stable for 36 months at 25°C (±2°C) and 60% (±5%) relative humidity and for 6 months at 40°C (±2°C) and 75% (±5%) relative humidity.

Expected toxicities:

Myelosuppression, nausea/vomiting, diarrhea, mucositis, skin rash (particularly hand-foot syndrome), fatigue, mental status changes/coma, allergic reactions (including fever, muscle aches, edema, dyspnea), congestive heart failure, conjunctivitis, anorexia, febrile neutropenia, pruritus, headache, flushing and pyrexia, hepatic enzyme elevations, liver failure.

5. Filgrastim-sndz (Zarxio, G-CSF)

THERAPEUTIC CLASSIFICATION: Recombinant growth factor

MECHANISM OF ACTION: Filgrastim, filgrastim-sndz, and tbo-filgrastim are granulocyte colony stimulating factors (G-CSF) produced by recombinant DNA technology. G-CSFs stimulate the production, maturation, and activation of neutrophils to increase both their migration and cytotoxicity.

PHARMACEUTICAL DATA: SC Injection-IV Infusion. Commercially available in single-dose, preservative-free vials containing 300 mcg (1 mL, 1.6 mL) and 480 mcg (0.8 mL) of Filgrastim-sndz. Used portions should be discarded.

STORAGE AND STABILITY: Filgrastim-sndz should be stored at 2° to 8° C. Prior to injection, Filgrastim-sndz may be allowed to reach room temperature, however, any vials left at room temperature for greater than 24 hours should be discarded. Vials should not be shaken. Vials should be inspected for sedimentation or discoloration prior to administration. If sedimentation or discoloration is observed, the vials should not be used.

KNOWN SIDE EFFECTS: Mild to moderate bone pain is possible in patients receiving myelosuppressive therapy. General skin rash, alopecia, fevers, thrombocytopenia, osteoporosis, nausea, vomiting, diarrhea, mucositis, anorexia, inflammation of the blood vessels, and/or cardiac dysrhythmia can occur. Splenomegaly may result at high doses of Filgrastim-sndz.

6. Fludarabine

THERAPEUTIC CLASSIFICATION: Antineoplastic fluorinated nucleoside analog

MECHANISM OF ACTION: After phosphorylation to fluoro-ara-ATP the drug appears toincorporate into DNA and inhibit DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis.The half-life of the activated compound is approximately 10 hours, but the pharmacology is incompletely understood. Excretion is impaired in patients with impaired renal function.

PHARMACEUTICAL DATA: Each vial contains 50 mg lyophilized drug, to be reconstituted with 2 ml sterile water to a solution that is 25 mg/ml for IV administration. Solution Preparation: mix each vial with 2 ml sterile pyrogen-free water to a clear solution, which is 25 mg/ml for IV administration only.

STABILITY AND STORAGE: Reconstituted solution should be used within 8 hours.
KNOWN SIDE EFFECTS: pancytopenia, immunosuppression, autoimmune hemolytic anemia has (rarely) been reported, and recurred when patients were retreated with the drug. Nausea, vomiting, anorexia, weakness. From the CNS: agitation, visual disturbances, confusion, coma, peripheral neuropathies have been reported. With high dose use confusion, blindness, coma and death have been reported.

SPECIAL PRECAUTIONS: for other antineoplastic agents Fludarabine should be handled by trained personnel using procedures for proper handling. The use of gloves and protective glasses is recommended to avoid exposure upon accidental spillage.

7. Melphalan

THERAPEUTIC CLASSIFICATION: Antineoplastic alkylating agent

MECHANISM OF ACTION: Interferes with DNA replication and transcription of RNA and ultimately results in the disruption of nucleic acid function.

PHARMACEUTICAL DATA: Supplied as 50 mg vials, reconstitute in 10 ml of diluent
STORAGE AND STABILITY: Reconstituted solution retains 90% potency for about 3 hours at 30º C. Storage at 5º C results in precipitation. Intact packages can be stored at room temperature.

KNOWN SIDE EFFECTS: Dose limiting toxicity is hematologic. GI toxicity is infrequent and mild including; nausea, vomiting, stomatitis, abdominal cramping and diarrhea. Azotemia has been reported. Alopecia and hypersensitivity reactions when high-doses are utilized.

8. Mycophenalate Mofetil (MMF)

THERAPEUTIC CLASSIFICATION: Immunosuppressant for GVHD prophylaxis
MECHANISM

OF ACTION: MPA inhibits T and B cell proliferation by blocking the

production of guanosine nucleotides required for DNA synthesis.

PHARMACEUTICAL DATA: Oral: 250-mg and 500-mg capsules, and as 200 mg/mL oral suspension. It may also be prepared extemporaneously as an oral suspension (100 mg /mL; in cherry syrup; pH = 6.0 - 6.7; Injectable: 500 mg vial to be reconstituted in 14 mL of D5W to yield about 15 mL containing 33.33 mg/mLof MMF.

STABILITY AND STORAGE: Oral: stable for at least four months at room temperature. Injectable: Each 500-mg vial should be reconstituted with 14 mL D5W to yield about 15 mL of MMF solution (33.33 mg/mL) as above. The dose should be further diluted in D5W to a final concentration of about 6 mg/mL. The solution should not be refrigerated and should be administred within 4 hrs of preparation. The dose should be infused over at least two hrs. The manufacturer does not specify the type of container to use for parenteral MMF.

KNOWN SIDE EFFECTS: constipation, diarrhea, nausea, vomiting, headache, confusion, tremor, gastrointestinal bleeding, hypertension, peripheral edema, cough exacerbation, infection, sepsis and bone marrow suppression including severe neutropenia.

9. Rituximab

THERAPEUTIC CLASSIFICATION: A genetically engineered chimeric/murine monocolonal antibody

MECHANISM OF ACTION: The Fab domain of rituximab binds to the CD20 antigen on the B lymphocytes, and the Fc domain recruits immune effector functions to mediate B cell lysis in vitro. Possible mechanisms of cell lysis include complement-dependent cytotoxicity (CDC) and antibody-dependent cell mediated toxicity (ADCC). The antibody has been shown to induce apoptosis in the DHL-4 human B cell lymphoma line.

PHARMACEUTICAL DATA: Rituximab is diluted in 5% Dextrose in Water or 0.9% NaCl to a final concentration of 1-4mg/ml and administered as an IV infusion (see section 5, treatment schedule). It should not be administered as a IV bolus. It s supplied as 100 mg and 500 mg sterile, preservative free, single use vials.

STORAGE AND STABILITY: Rituximab vials should be stored at 2-8 C. Vials should be protected from direct sunlight. Diluted rituximab solution is stable at 2-8 C for 24 hours and at room temperature for an additional 12 hours.

KNOWN SIDE EFFECTS: Rituximab is associated with a infusion related symptom complex comprising of fever and chill/rigors. Other frequent infusion-related symptoms include nausea, urticaria, fatigue, headache, pruritis, bronchospasm, dyspnea, angioedema, rhinitis, vomiting, hypotension, flushing and pain at disease sites.

10. Tacrolimus

THERAPEUTIC CLASSIFICATION**:** A cyclic polypeptide immunosuppressive.

MECHANISM OF ACTION: Macrolide antibiotic produced by Streptomyses tsukubaensis that inhibits T and B cell proliferation.

PHARMACEUTICAL DATA: Available in 5mg/mL vials. Anhydrous is diluted in alcohol dehydrated 80% v/v and polyoxyl 60 hydrogenated castor oil 200 mg/mL. For IV infusion, solution is diluted with 0.9% sodium chloride or 5%dextrose to a concentration of 4-20 ug/mL. Also available in tablets; 0.5, 1, and 5 milligrams. Disposition is biphasic with a terminal half life of 10-30 hours. Elimination is mainly by hepatic metabolism and biliary excretion.

STORAGE AND STABILITY: The oral form is stable at room temperature protected from light for at least 2 months. The IV preparation is diluted in D5W or NS and is stable for 24 hours.

KNOWN SIDE EFFECTS: renal failure, hepatic toxicity, hypertension, nausea, headache, confusion, tremor, seizures, peripheral edema, infection, sepsis and bone marrow suppression

11. Total Body Irradiation (TBI)

TBI may cause low blood cell counts (white blood cells, red blood cells, and platelets). During

treatment there is an increased risk of infection, including pneumonia. Other side effects include anemia, abnormal bleeding, bruising, fatigue, and/or shortness of breath. A blood transfusion may be required.

TBI may cause nausea, vomiting, mouth sores, diarrhea, esophageal pain, liver damage from veno-occlusive disease, and/or hair loss. It may cause cataracts, chronic injury to the lungs resulting from increased fibrosis, and/or destruction of lung tissue. It may cause an increased risk of developing other cancers.

TBI may damage the heart and/or the lungs, which may result in shortness of breath. TBI may also cause swelling of and bleeding from the bladder, hepatitis, and/or abnormal levels of minerals in the blood. It may cause dry mouth and/or changes in taste.

Fucosylation Reagents

12. Alpha 1,3 fucosyltransferase-VI (FTVI)

THERAPEUTIC CLASSIFICATION: Adhesion molecule (selectin ligand) modulator

MECHANISM OF ACTION: FTVI is a glycosyltransferase that modifies terminal sugars known as “type 2 sialyllactosamine units”, thereby engendering expression of an oligosaccharide known as sialyl Lewis X (sLeX; CD15s). The sLeX motif is the principal E-selectin binding determinant, and is displayed on the CD44, PSGL-1, and CD43 glycoproteins of human CD34+ hematopoietic stem/progenitor cells (HSPCs). Expression of sLex mediates binding of human HSPCs to E-selectin-bearing bone marrow microvascular cells. This adhesive interaction results in homing of human HSPCs to bone marrow.

PHARMACEUTICAL DATA: FTVI (supplied by Warrior Therapeutics) is provided as a frozen sterile solution in a vial containing 452 ug enzyme (at concentration of 1.19 mg/mL) in 5% glycerol and 95% Hank’s Balanced Salt Solution without calcium/magnesium (i.e., HBSS without Ca/Mg). Each vial will contain requisite enzyme to achieve fucosylation of human umbilical cord blood cells suspended in a 50 ml reaction volume. Upon thawing, FTVI is a clear, colorless solution, visibly free of particulate material. FTVI is a reagent, used ex vivo as a critical component in the enzymatic fucosylation processing of human umbilical cord blood stem cells for infusion.

STORAGE AND STABILITY: FTVI should be stored at -80°C (-70°C to -80°C). Once thawed, it should be used promptly in the fucosylation reaction. Fucosylated stem cells

are stable for up to 2 hours at room temperature. Unused or expired FTVI will be destroyed per institutional policy.

KNOWN SIDE EFFECTS: None.

13. Guanosine-5'diphospho-β-L-fucose (GDP-fucose)
THERAPEUTIC CLASSIFICATION: Adhesion molecule (selectin ligand) modulator
MECHANISM OF ACTION: GDP-fucose is the donor nucleotide sugar substrate utilized by FTVI in the aforementioned enzymatic reaction, thereby providing the needed missing fucose on the terminal type 2 sialyllactosamine unit(s) to create sLeX.,

PHARMACEUTICAL DATA: GDP-fucose (supplied by Warrior Therapeutics) is provided as a sterile powder of white appearance in vials packaged in amount of 31.7 mg, requisite to achieve a final concentration of 1 mM GDP-fucose in 50 ml reaction volume. For enzymatic reactions, it is reconstituted in sterile HBSS without Ca/Mg, with the appearance of clear, colorless solution, visibly free of particulate material.
GDP-fucose is a reagent, used ex vivo as a critical component in the enzymatic fucosylation processing of human umbilical cord blood stem cells for infusion.

STORAGE AND STABILITY: GDP-fucose powder is stable at room temperature and in the cold (i.e., it can be stored in frost-free freezers). Once reconstituted into solution, it should be used promptly in the fucosylation reaction. Fucosylated stem cells are stable for up to 2 hours at room temperature. Unused or expired GDP-fucose will be destroyed per institutional policy.

KNOWN SIDE EFFECTS: None.

4.0 Patient Eligibility

Inclusion Criteria

1. Patients must have one of the following hematologic malignancies:

    a. Acute Myelogenous Leukemia (AML), induction failure, high-risk for relapse first remission (with intermediate-risk or high-risk cytogenetics, flt3 mutation positive and/or evidence of minimal residual disease by flow cytometry), secondary leukemia from prior chemotherapy and/or arising from MDS, Langerhan's cell histiocytosis, any disease beyond first remission.

    b. Myelodysplastic Syndrome (MDS): MDS IPSS INT-1 will be enrolled only if the subjects have failed previous leukemia treatments and are transfusion-dependent. MDS may be primary or therapy related, including patients that will be considered for transplant. Including the following categories: 1) revised IPSS intermediate and high risk groups, 2) MDS with transfusion dependency, 3) failure to respond or progression of disease on hypomethylating

agents, 4) refractory anemia with excess of blasts, 5) transformation to acute leukemia, 6) chronic myelomonocytic leukemia, 7) atypical MDS/myeloproliferative syndromes, 8) complex karyotype, abn(3g), -5/5g-, -7/7g-, abn(12p), abn(17p).

c. Acute Lymphoblastic Leukemia (ALL) patients with the following will be considered: Induction failure, primary refractory to treatment (do not achieve complete remission after first course of therapy) or are beyond first remission including second or greater remission or active disease. Patients in first remission are eligible if they are considered high risk, defined as any of the following detected at any time: with translocations 9;22 or 4;11, hypodiploidy, complex karyotype, secondary leukemia developing after cytotoxic drug exposure, and/or evidence of minimal residual disease, or acute biphenotypic leukemia which includes > 7 chromosomal abnormalities, or double hit non-Hodgkin's lymphoma.

d. Non-Hodgkin's Lymphoma (NHL) in second or third complete remission or relapse (including relapse post autologous hematopoietic stem cell transplant), or relapsed double hit lymphoma.

e. Small Lymphocytic Lymphoma (SLL), or Chronic Lymphocytic Leukemia (CLL) with progressive disease with progression after standard of care therapy orhave failed/been intolerant to ibrutinib.

f. CML second chronic phase or accelerated phase.

g. Hodgkin's Disease (HD): Induction failure after the first complete remission, or relapse (including relapse post autologous hematopoietic stem cell transplant), or those with active disease.

2. The first 6 patients must be ≥ 18 and ≤ 65 years old. The subsequent patients may include pediatric patients ≥ 12 and ≤ 65 years old. Eligibility for pediatric patients will be determined in conjunction with an MDACC pediatrician.
3. Performance score of at least 80% by Karnofsky or PS < 3 (ECOG) (age ≥ 12 years).
4. Adequate major organ system function as demonstrated by:
   a. Left ventricular ejection fraction of > 40%
   b. Pulmonary function test (PFT) demonstrating a diffusion capacity of least 50% predicted.
   c. Creatinine ≤ 1.5 mg/dL.
   d. SGPT/bilirubin ≤ to 2.0 x normal.
5. Negative Beta HCG test in a woman with child bearing potential defined as not post-menopausal for 12 months or no previous surgical sterilization and willing to use an effective contraceptive measure while on study.
6. Patients must have two CB units available which are matched with the patient at 4, 5, or 6/6 HLA class I (serological) and II (molecular) antigens. Each cord must

contain at least 1.5 x 10^7 total nucleated cells/Kg recipient body weight (pre-thaw).
7. Patient must not have a 10/10 HLA matched family member or unrelated donor.
8. Patients will have a back-up graft from any of the following: an available fraction of autologous marrow; or PBPCs harvested and cryopreserved; or family member donor; or a third cord blood unit.
9. Prior to initiating chemotherapy in this study, twenty-one or more days must have elapsed since the patient's last radiation or chemotherapy administration (Hydrea, Gleevec and other TKI inhibitors as well as intra-thecal therapy are accepted exceptions).
10. Patients enrolled in this study may be on other IND studies at the discretion of the PI.

Patient Exclusion Criteria

1. Patients with known history of HIV/AIDS.
2. Patients with positive hepatitis serology that is definitive of active disease.
3. Active CNS disease in patient with history of CNS malignancy.
4. Patients with chronic active hepatitis or cirrhosis. If positive hepatitis serology, the Study Chair may deem the patient eligible based on the results of liver biopsy.
5. Patients with uncontrolled serious medical condition such as persistent septicemia despite adequate antibiotic therapy, decompensated congestive heart failure despite cardiac medications or pulmonary insufficiency requiring intubation (excluding primary disease for which CB transplantation is proposed), or psychiatric condition that would limit informed consent.
6. Pediatric patients with acute lymphoblastic leukemia (ALL) that is t (9,22) positive in first remission are not eligible unless there is evidence of minimal residual disease after initial induction and/or consolidation treatment or the pediatric Ph+ ALL is clinically refractory to available therapies with evidence of persistence in the bone marrow or peripheral blood.
7. Patients with options for treatment that are known to be curative are not eligible.

5.0 Treatment Plan

The investigational component of the treatment plan of this study is the infusion of one MPC expanded and one fucosylated CB unit.

Enrollment of the first 3 participants will be staggered by at least 30 days. The occurrence of two or more subjects with Grade 3 or 4 toxicities considered related or one death considered related within 30 days following stem cell infusion will stop further enrollment of study subjects and treatment with study agent pending investigation of the risk by the MDACC IRB and the FDA.

Participant data will be collected in CORe and PDMS. A cohort summary must be completed and submitted to the IND Office Medical Monitor after the 3rd subject reaches 42 days post infusion, after the 4th subject reaches 42 days post infusion, and every 4 subjects thereafter.

Preparative regimens used in this study are commonly used for CB transplantation. The selection of these will depend on patient's age, disease and co-morbid condition. Prior to initiating chemotherapy in this study, twenty-one or more days must have elapsed since the patient's last radiation or chemotherapy administration (Hydrea, Gleevec and other TKI inhibitors as well as intra-thecal therapy are accepted exceptions). Patients will have a back-up graft from any of the following: an available fraction of autologous marrow; or PBPCs harvested and cryopreserved; or family member donor; or a third cord blood unit.

Study Design

Once consented, the CB units will be shipped to MD Anderson. The CB unit which is the least well HLA-matched will be expanded with MPC while the better HLA-matched CB unit will be fucosylated. If both CB units have the same HLA-match, the smaller unit (lower total nucleated cell dose) will be expanded and larger fucosylated.

Patients will be admitted to the hospital on day -11, -10, or -9 for hydration and will receive the designated preparative regimen as described in Preparative Regimens, below.

The CB unit to be expanded will be thawed and undergo *ex vivo* expansion by co-culture on MPCs. Four to seven days prior to the thawing and washing of the CB unit that will undergo expansion, a container of MPCs is thawed and expanded into culture flasks. The CB unit to undergo expansion is evenly divided among 10 flasks of MPCs for the co-culture expansion. After 7 days of co-culture of the CB cells on the MPC adherent cells, the non- adherent CB cells are collected from the flasks containing the MPCs and transferred to cell culture bags. Culture of any residual CB cells remaining in the MPC flasks is also continued. Cells in both bags and flasks are cultured expanded for an additional 7 days. After a total of 14 days in culture, the non-adherent CB stem cells from both the bags and flasks are pooled together. The other CB unit will be incubated with GDP β-fucose and the enzyme fucosyltransferase-VI (FT-VI) for up to 60 minutes. After incubation, the CB cells will be washed and immediately infused.

Unmanipulated Cord Blood: The unmanipulated CB units are obtained from the National Marrow Donor Program, Netcord, and other suitable registries and are matched with the subject at 4, 5, or 6/6 HLA class I (intermediate resolution) and II (high resolution) loci.

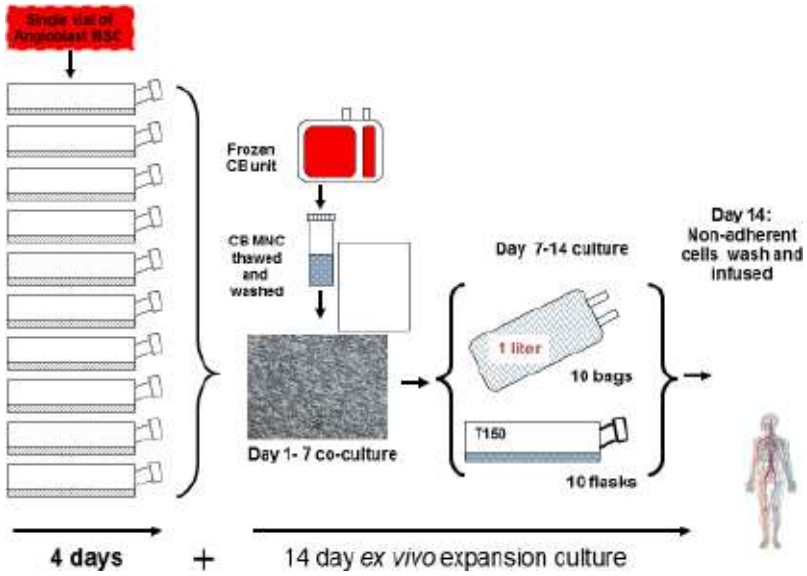
On day 0, the CB unit will be thawed, fucosylated, washed, and infused, followed by the infusion of the MPC-expanded CB unit. The infusions will be separated by a minimum of 30 minutes.

Both CB units will be administered with the use of a standard Y-type blood/solution set with large blood filter 170 to 260 micron filter by intravenous infusion (IV) via gravity. CB

units are not to be infused with the use of an infusion pump. The rate of administration of the CB units is not to exceed 250 mL/hour (maximum infusion rate).

Unused or expired fucosylation agents will be used for validation experiments or destroyed per institutional policy.

Figure 9 Study Design



Preparative Regimens

Chemotherapy agent doses and administration.

Below are the regimens combination, doses and schedule.

Patients with B cell malignancies will receive Rituximab (or a biosimilar of rituximab).

ATG is dosed per actual body weight.

Clofarabine and fludarabine are dose adjusted per body weight if patient is >20% expected weight.

Rituximab is dosed per actual body weight.

Fludarabine/Clofarabine/Busulfan/TBI (Myeloablative Regimen)

Recommended for patients who are <55 years old. Patients > 55 but < 65 years who have a Performance Status of 0 or 1 and no comorbidities may receive the myeloablative regimen at the discretion of the investigator(s).

Flu-Clo-Bu-TBI

Flu = Fludarabine

Clo = Clofarabine

Bu = Busulfan

ATG = Rabbit Antithymocyte Globulin

TBI = Total Body Irradiation (low dose)

Patients will receive Busulfan as per standard of care. Busulfan test dose can be administered either as an outpatient prior to admission or as an inpatient on Day -10. Busulfan pharmacokinetics will be performed with the test dose and the first dose on Day -7 per standard of care. The doses of Days -6, -5, and -4 will be subsequently adjusted to target an AUC of 4,000 microMol.min-1. In the event that PK adjusting were not possible a dose of busulfan of 130 mg/m2 will be administered.

All patients will receive echinocandins for fungal prophylaxis until patient engrafts neutrophils unless prior infectious history dictates an azole.

Inpatient Busulfan Test Dose Treatment Plan:

Day	Treatment
-20 (approximate)	MPC start
-14	Cord culture
-11	Admit / IV Hydration / Rituximab 375 mg/m IV (B cell malignancy)*
-10	Test dose Bu 32 mg/m^2 (PK sampling)
-9	Rabbit ATG 1.25 mg/Kg IV
-8	Rabbit ATG 1.75 mg/Kg IV
-7	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000 (PK sampling)
-6	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000
-5	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000
-4	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000
-3	Low-Dose TBI 2 Gy in AM
-2	Rest
-1	Rest
0	Cord Blood Infusions

* A biosimilar of rituximab may be used.

Outpatient Busulfan Test Dose Treatment Plan:

Day	Treatment
	Outpatient test dose BU 32 mg/m^2 (PK Sampling)
-20 (approximate)	MPC start
-14	Cord culture
-10	Admit / IV Hydration / Rituximab 375 mg/m (B cell malignancy)*
-9	Rabbit ATG 1.25 mg/Kg IV
-8	Rabbit ATG 1.75 mg/Kg IV
-7	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000 (PK Sampling)
-6	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000
-5	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000
-4	Flu 10 mg/m^2 / Clo 30 mg/m^2 / Bu AUC 4,000
-3	Low-Dose TBI 2 Gy in AM
-2	Rest
-1	Rest
0	Cord Blood Infusions

* A biosimilar of rituximab may be used.

Fludarabine/Melphalan (Reduced Intensity)

Recommended for patients of any age that in the opinion of the investigator(s) would preclude myeloablative therapy.

DAY	TREATMENT
-20 (approximate)	MPC start
-14	Cord culture
-9	Admit/Hydration
-8	Rabbit ATG 1.25 mg/Kg IV
-7	Rabbit ATG 1.75 mg/Kg IV
-6	Rest
-5	Fludarabine 40 mg/m^2 IV
-4	Fludarabine 40 mg/m^2 IV
-3	Fludarabine 40 mg/m^2 IV
-2	Fludarabine 40 mg/m^2 IV / Melphalan 140 mg/m IV
-1	Rest
0	Cord Blood infusions

Fludarabine/Cyclophosphamide/TBI 200

Recommended for patients of any age that in the opinion of the investigator(s) would preclude myeloablative therapy.

DAY	TREATMENT
-20 (approximate)	MPC start
-14	Cord culture
-9	Admit/Hydration
-8	ATG 1.25 mg/kg IV
-7	ATG 1.75 mg/kg IV
-6	Fludarabine 40mg/m^2 IV / Cyclophosphamide 50 mg/kg IV / Mesna 50 mg/kg IV
-5	Fludarabine 40mg/m^2 IV
-4	Fludarabine 40mg/m^2 IV
-3	Fludarabine 40mg/m^2 IV
-2	Rest
-1	TBI 200 cGy
0	Cord Blood infusions

GVHD Prophylaxis and Supportive Care as per standard practice in patients receiving allogeneic transplant and SCTCT Guidelines

Graft Versus Host Disease prophylaxis with Tacrolimus and MMF. Tacrolimus starting dose 0.03 mg/kg or 0.015 mg/kg (ideal body weight) IV starting on D -2 and will be tapered around D+180 if no GvHD is present. Mycophenolate (MMF) will be dosed at 15mg/kg (actual body weight with a maximum dose of 1 gram twice daily) IV or PO administered from days D -3 to D +100 in the absence of GvHD. MMF can be adjusted based on renal function.

Filgrastim-sndz dose 5 micrograms/kg/day SQ rounded to the nearest vial starting on

Day 0 until engraftment. For Pediatric dosing, 5 micrograms/kg/day, dose may be increased to 10mcg/kg/day starting on Day +21. Pediatric dosing should not be rounded to the nearest vial size, unless indicated by treating physician.

Infectious disease prophylaxis, blood and platelet transfusion and other supportive treatment as clinically indicated.

Birth Control

All participants who are able to have children must practice effective birth control while on study. Acceptable forms of birth control for female patients include: hormonal birth control, intrauterine device, diaphragm with spermicide, condom with spermicide, or abstinence, for the length of the study. If the participant is a female and become pregnant or suspects pregnancy, she must immediately notify her doctor. If the participant becomes pregnant during this study, she will be taken off this study. Men who are able to have children must use effective birth control while on the study. If the male participant fathers a child or suspects that he has fathered a child while on the study, he must immediately notify his doctor.

Concurrent Treatment

Patients that develop life-threatening graft versus host disease, veno-occlusive disease, cytomegalovirus, adenovirus, Epstein Barr, or BK viral infections may be enrolled on an investigational treatment study with the approval of the principal investigator.

6.0 Pretreatment evaluation

The following evaluation includes standard tests performed to determine patient eligibility for transplant and are not required to be repeated prior to starting treatment unless treatment is delayed for more than 30 days after registration.

1. History and physical examination.
2. CBC, differential and platelet count.
3. BUN, creatinine, bilirubin, alanine aminotransferase, aspartate aminotransferase.
4. Infectious disease screening, which will include testing for HIV, Hepatitis B, Hepatitis C**,** West Nile Virus, HTLV, TPHA, and CMV.
5. Chest X-Ray, mini sinus CT.
6. EKG.
7. Echocardiogram or MUGA scan.
8. Pulmonary function tests with DLCO.
9. Bone marrow aspirate with cytogenetics (not required if patient has circulating blasts and known cytogenetic results).
10. HLA class I and II typing.
11. Beta HCG for women of child bearing potential (not postmenopausal for 12 months; no prior surgical sterilization).

7.0 Evaluation During Study

Post transplant Evaluations:

To be performed at approximately 30 days, 3, 6, and 12 months post transplant. These evaluations follow our standard practice and are done to determine engraftment and disease status. If clinically indicated these studies may be done at other time points which can replace the nearest planned timepoint.

1. Chimerism studies from peripheral blood performed on separated T-cells and myeloid cells.
2. At each visit, a physical examination and adverse event documentation including GvHD assessment.
3. The following lab tests are to be performed as frequently as clinically indicated: CBC, differential, platelets, SGPT, calcium, glucose, uric acid, magnesium, serum bilirubin, BUN and creatinine, serum protein, albumin, alkaline phosphatase, electrolytes, urinalysis, tacrolimus levels and CMV antigenemia.
4. Disease specific assessment as per standard of care and the Stem Cell Transplantation and Cellular Therapy Department guidelines.

Additional lab tests:

Additional lab draws may be requested to monitor the cord blood units and patient disease status. The patient may be asked to sign a separate consent for protocol LAB00-099 for these additional samples. Analysis will be performed in Dr. Katy Rezvani's Lab free of charge for the patient. Analysis results from these samples performed under protocol LAB00-099 will be used in protocol 2016-0051.

8.0 Statistical Considerations

This is a phase 2 clinical trial in which patients with hematologic malignancies following high-dose therapy will receive one MPC expanded CB unit plus one fucosylated CB unit in the DUCBT setting. The CB unit which is the least well HLA-matched will be expanded with MPC co-culture while the better HLA-matched CB unit will be fucosylated. If both CB units have the same HLA-match, the smaller unit (lower total nucleated cell dose) will be expanded for 14 days while the larger will be fucosylated with FTVI and GDP-fucose both as previously described in the body of this protocol. CB infusion of the fucosylated unit is to take place on day 0 after infusion of the first, unmanipulated CB unit.

We define feasibility as having a sterile expanded MPC product and/or fucosylation a >50% fucosylated unit in the majority of the patients enrolled.

Enrollment of the first 3 participants will be staggered by at least 30 days. The occurrence of two or more subjects with Grade 3 or 4 toxicities considered related or one death considered related within 30 days following stem cell infusion will stop further

enrollment of study subjects and treatment with study agent pending investigation of the risk by the MDACC IRB and the FDA.

***Monitoring Time to Engraftment*.** The distribution of time to engraftment will be estimated using Kaplan-Meier plots and the relationship to covariates evaluated by piecewise exponential Bayesian regression. Time to engraftment will be monitored by the Bayesian statistical method of Thall et al., assuming an accrual rate of 10 patients per year with a maximum sample size of 25 patients. A mean time to engraftment (TTE) of 7 days will be considered desirable and a mean TTE of 21 days will be considered undesirable. The design is based on the assumptions that mS = standard (historical) mean time-to-engraftment follows an informative Inverse Gamma (51, 350) prior, with a mean of 7 days and variance of 1, and that mE follows a non-informative Inverse Gamma (2.49, 10.43) prior, which also has a mean of 7 days but a much larger variance of 100. The possibly right-censored times time-to-engraftment will be monitored continuously throughout the trial. Accrual will be terminated due to futility if at any time during the trial Prob(mE < mS | data) < .0985. This cut-off was chosen to ensure a small early stopping probability if the true mean time to engraftment = 7 days (median = 4.85 days). The operating characteristics of this monitoring rule were computed using the program TTEDesigner version 1.2.2, available from the MDACC Department of Biostatistics (**Table 3**). The trial will be conducted using the Clinical Trials Data Monitoring website maintained by the Department of Biostatistics at MDACC.

Table 3. Operating characteristics of the design for monitoring time-to-engraftment, with maximum samples size 25.

True Mean Time to Engraftment (days)	True Median Time to Engraftment (days)	True Prob of Engraftment within 7 days	Pr (Stop Early)	Mean Sample Size
7	4.85	0.37	0.14	22.8
21	14.56	0.05	1.0	4.0

Infusion-related mortality (IRM) is defined as death within 24 hours that is definitely related to the stem cell infusion. Accrual will be terminated if any IRM is observed.

Monitoring GVHD. GVHD4 = grade 4 graft-versus host disease within 42 days will be monitored using the Bayesian statistical method of Thall and Sung (1998), Denoting pE = Pr(GVHD4 with the experimental therapy), the historical value .20 will be considered acceptable and .40 unacceptable. Assuming that pE follows an uninformative beta(.20,.80) prior, and that the historical probability pS follows an informative beta(200,800) prior, accrual will be terminated early if Pr(pS < pE | data) > .95. Applying this rule after each cohort of 4 patients, the trial will be stopped if [# patients with GVHD4] / [# patients evaluated] is greater than or equal to 3/4, 4/8, 6 /12, 7/16, 8 /20 or 9/24. For (undesirably high) true p = .40, this rule has early stopping probability is .75, with sample size quartiles (8, 16, 25). For (acceptably low) true p = .20, the early stopping probability is .10 with sample size quartiles (25, 25, 25).

Secondary outcomes include disease-free survival, TRM, and OS.

The distributions of disease-free survival and overall survival time will be estimated using Kaplan-Meier plots and their relationships to covariates evaluated by piecewise exponential Bayesian regression. TRM will be evaluated by tabulation, Kaplan-Meier plots, and piecewise exponential Bayesian regression. TRM will be evaluated by tabulation, Kaplan-Meier plots, and piecewise exponential Bayesian regression.

9.0 Criteria for Response

Disease Response Is defined according to disease specific criteria as per the CIBMTR.

Engraftment is defined as the evidence of donor derived cells (more than 95%) by chimerism studies in the presence of neutrophil recovery by day 28 post cord blood infusion.

Other definitions used to assess engraftment:

Neutrophil recovery is defined as a sustained absolute neutrophil count (ANC) > 0.5 x 10^9/L for 3 consecutive days.

Engraftment date is the first day of three (3) consecutive days that the ANC exceeds 0.5 x10^9/L.

Delayed engraftment is defined as the evidence of engraftment beyond day 28 post blood unit infusion achieved after the administration of therapeutic (high dose) hematopoietic growth factors.

Primary Graft failure is defined as failure to achieve an ANC > 0.5 x 10^9/L for 3 consecutive days by day 28 post cord blood infusion, with no evidence of donor derived cells by bone marrow chimerism studies and no evidence of persistent or relapsing disease.

Secondary graft failure is defined as a sustained declined of ANC <0.5 x 10^9/L for 7 consecutive days unresponsive to hematopoietic growth factors in the absence of relapse after initial documented engraftment or loss of donor chimerism in the absence of leukemic relapse.

Autologous reconstitution is defined by the presence of ANC > 0.5 x 10^9/L without evidence of donor-derive cells by bone marrow chimerism studies. This can occur at initial engraftment or later after initial engraftment has been documented.

Treatment of Engraftment Failure. Will be further defined according to patient's condition and type of graft available following the SCT&CT guidelines for treatment of graft failure.

10.0 Criteria for Removal from the Study

Any patient can be removed from study for the following:

- Patient withdrawal of the informed consent
- Patient not being compliant
- An increasing or unexpected pattern of toxicity is observed deemed unacceptable by the study chairman
- Disease progression
- Investigator judgment when the well being and best interest of the patient is compromised
- Primary or Secondary Graft Failure
- Death
- One year from treatment completion

11.0 Adverse Events and Reporting Requirements

Assessment of the Adverse Events Severity

The severity of the adverse events (AEs) ≥ Grade 3 will be graded according to the Common Terminology Criteria v4.0 (CTCAE) from the start of preparative regimen up to D+100. Events not included in the CTCAE chart will be scored as follows:

General grading:

Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.

Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.

Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.

Grade 4: Life Threatening: discomfort that represents immediate risk of death

Grading for specific syndromes: Veno-occlusive disease (VOD):

Grade 3: Bili >2mg/dl with at least two of the following: increased weight >4% from

baseline, ascites or hepatomegaly

Grade 4: pulmonary and or renal failure

Pulmonary events not caused by CHF (interstitial pneumonitis (IP), pulmonary hemorrhage (DAH):

Grade 1: CXR showing mild infiltrates or interstitial changes Grade 2: mild SOB

Grade 3: requires supplemental oxygen, or is a documented infection Grade 4: requires intubation

Thrombotic thrombocytopenic purpura (TTP): Grade 1: No treatment required

Grade 2: Requires steroids and/or plasma transfusions Grade 3: Requires plasma exchange

Cytokine storm or engraftment syndrome: Grade 1: No treatment required Grade 2: Treatment required

Grade 3: Organ dysfunction Grade 4: Total Bilirubin >5

Hemorrhagic Cystitis:

Grade 1: minimal or microscopic bleeding/pain Grade 2: gross bleeding/pain and spasms

Grade 3: transfusion/irrigation required Grade 4: dialysis required

Causality Assessment

The investigational component of the treatment plan of this study is the infusion of an MPC-expanded, then fucosylated CB unit in conjunction with an unmanipulated CB unit.

The infusion of an MPC-expanded then fucosylated CB unit has not been fully tested in humans and the side effects are not well understood. Therefore, for the purpose of this study when in the presence of an adverse event which a direct relationship to the fucosylated CB unit is supected, the casualty of the event will be attributed to the fucosylation plus MPC expansion.

In this study the treatment plan (preparative regimen followed by infusion of both CB) is defined as the “transplant package”. Therefore, events known to be caused by components of the transplant package and its direct consequences as well as those events known to be related to drugs used for the treatment of GVHD, infections and supportive treatment will be scored as unrelated to the fucosylation.

The principal investigator will be the final arbiter in determining the casualty assessment.

List of most common expected adverse events

1. Infections in the presence or absence of neutropenia: fungal, bacterial and or viral infections.
2. Fever: Non-neutropenic or neutropenic without infection
3. Acute graft versus host disease (aGVHD): most commonly manifested by skin rash, diarrhea and abnormal liver function tests could also present with some degree of fever, upper gastrointestinal symptoms (nausea and vomiting) mucositis and eye dryness.
4. Gastrointestinal (GI tract): the GI tract manifestations could be not only due to direct damage from the preparative regiment but also be a manifestation of GVHD or infections. Therefore, the time, course, and its presentation are crucial when assessing these as adverse events. Nausea/vomiting, mucositis, and diarrhea when presented within first 7 to 10 days most likely will be related to the preparative regimen.
5. Skin rash: not related to GVHD could be caused by chemotherapy used for the preparative regimen or antibiotics used as supportive treatment.
6. Transaminitis: liver function test elevation.
7. Pulmonary events: not related to CHF most likely caused by drug injury or infection. These could present with a pneumonitis pattern manifested with shortness of breath, pulmonary infiltrates on chest radiograph, sometimes accompanied by fever and cough and progress to acute respiratory insufficiency and a diffuse bilateral alveolar pattern.
8. Cytokine Storm/ engraftment syndrome: most likely caused by released cytokines.
9. Hemorrhagic cystitis: not related to chemotherapy agents used in the proposed preparative regimen is most likely caused by viral infection.
10. Thrombotic thrombocytopenic purpura (TTP).
11. Veno-occlusive Disease of the Liver (VOD): known to have a higher incidence in patients undergoing high dose chemotherapy. Some antimicrobial agents have been also incriminated in its development.
12. Fluid overload due to hydration required for conditioning regimen, blood product transfusions and or IV alimentation
13. Graft failure.
14. Chronic GVHD.
15. For the purpose of this study the following events would not be considered adverse events and would not be recorded in the database:
    1. Flu-like symptoms not associated with infection
    3. Abnormal laboratory findings considered associated to the original disease
    4. Isolated changes in laboratory parameters such as electrolyte, magnesium and metabolic imbalances, uric acid changes, elevations of ALT, AST, LDH and alkaline phosphatase.

Adverse events considered serious

1. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation).
2. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).
3. Graft Failure/ rejection.
4. Any expected or unexpected event resulting in an irreversible condition and/or leading to death.

Adverse events data collection

Adverse events will be collected in PDMS/CORe as applicable.

From the start of preparative regimen up to D+100 the collection of adverse events will reflect the onset and resolution date and maximum grade; beyond this point some events considered related to chronic GVHD or late complications post transplant might be recorded only with the first date of their awareness with no grade or resolution date. Intermittent events should be labeled as such and followed until resolution.

If a patient is taken off study while an event is still ongoing, this will be followed until resolution unless another therapy is initiated. Pre-existing medical conditions will be recorded only if an exacerbation occurs during the active treatment period. Co-morbid events will not be scored separately.

Adverse events will be documented based on progress notes, including the flowsheet, in the electronic patient medical record (EPIC).

PDMS/CORe will be used as the electronic case report form for this protocol and all protocol specific data will be entered into PDMS/CORe.

Concurrent medication

As stated in the treatment plan, patients treated on this protocol will require supportive care treatment (concurrent medications). These medications are considered standard of care and have no scientific contributions to the protocol, therefore no data will be captured on the various medications needed or their side effects.

Serious Adverse Events Reporting (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it

occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.
- Incidence of acute graft versus host disease (GVHD) ≥ Grade 3 over 50%.
- CTCAE grade ≥ 3 reactions related to the study cell infusion.
- CTCAE grade ≥ 3 organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) not preexisting or due to the underlying malignancy and occurring within 30 days of study product infusion.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- **Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.**
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in “The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices”. Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).
- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- **Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.**
- **Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.**
- **Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.**

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

12.0 References

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