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Cancer Experimental Therapeutics Initiative
Blood and Marrow Transplantation Program

Adoptive Transfer of T Regulatory Cell for Suppression of Acute Graft-vs-Host-Disease after an Umbilical Cord Blood Transplant for Hematologic Malignancies

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Protocol Synopsis

Adoptive Transfer of T Regulatory Cell for Suppression of Acute Graft-vs-Host-Disease after an Umbilical Cord Blood Transplant for Hematologic Malignancies

MT2016-17

Study Design:

This is a single center pilot study of a non-myeloablative umbilical cord blood transplant for the treatment of a hematological malignancy with a single infusion of T regulatory (Treg) given shortly after UCB transplantation..

The nTreg cells are manufactured from a cord blood unit that is not part of the graft for infusion on day 0 at least 1 hour after the UCB transplant. The CD3+ cell content from the graft UCB unit(s) is enumerated upon thaw (day 0) from which the Treg cell dose for the final product is calculated using assigned 1:1 Treg:CD3+ cell ratio.

Primary Objective:

To obtain preliminary estimates of efficacy as measured by length of Treg survival after infusion of Treg

Secondary Objectives:

To obtain preliminary estimates of safety as measured by

- Probability of grade II-IV aGVHD
- Probability of grade III-IV aGVHD
- Probability of treatment related mortality (TRM) at 6 months
- Probability of relapse at 1 year
- Probability of viral and fungal infections at 1 year
- The proportion of patients with detectable Treg cells at Day 14 post infusion
- Pattern of immune reconstitution
- Toxicity

Transplant Related Objectives:

- Determine the incidence of chimerism at Day 100
- Determine the probability of survival at 1 year
- Determine the incidence of neutrophil recovery at Day 42
- Determine the incidence of platelet recovery at 1 year
- Determine the incidence of chronic GVHD at 1 year

Age and UCB Graft Requirements

Must be ≥18, but < 70 years of age with no available medically suitable 7/8 or 8/8 HLA-matched sibling donor, considering HLA A, B, C and DRB1 - patients ≥ 70 and ≤ 75 years of age may be eligible if they have a Co-Morbidity score ≤ 2

Graft units will be selected according to the current University of Minnesota umbilical cord blood graft selection algorithm. The UCB unit that is not part of the graft will serve as the source of Treg cells.

Eligible Diseases:

- Acute Leukemias
- Burkitt's Lymphoma
- Natural Killer Cell Malignancies
- Chronic Myelogenous Leukemia
- Myelodysplastic Syndrome
- Large-Cell Lymphoma, Hodgkin Lymphoma, and Multiple Myeloma
- Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Marginal Zone B-Cell Lymphoma, Follicular Lymphoma:
- Lymphoplasmacytic Lymphoma, Mantle-Cell Lymphoma Prolymphocytic Leukemia

Refer to Section 5.2 of the protocol for complete disease information.

Other Inclusion Criteria:

- Karnofsky performance status of ≥ 70%
- Adequate organ function defined as:
  - Renal: creatinine ≤ 2.0 mg/dL, if > 1.2 mg/dL must have eGFR of ≥ 40
  - Hepatic: AST, ALT, and ALP ≤5 x upper limit of normal, total bilirubin ≤ 2.5 mg/dL
  - Pulmonary function: DLCO, FEV1, FVC ≥40% predicted and absence of O_2 requirements
  - Cardiac: Absence of decompensated congestive heart failure, or uncontrolled arrhythmia and left ventricular ejection fraction ≥ 40%
- Must have been exposed to highly immunosuppressive single agent or multi-agent chemotherapy within prior 3 months OR an ablative preparative regimen for autologous hematopoietic stem cell transplant within previous 1 year
- Voluntary written consent signed by the participant

Patient Exclusion:

- Untreated active infection at time of transplantation
- History of HIV infection
- Pregnant or breast feeding
- Prior allogeneic transplantation
- Less than 3 months from myeloablative conditioning for autologous transplantation

Treatment Plan: An 18 day lead time is required for the manufacturing of the Treg cells

A non-myeloablative cyclophosphamide/fludarabine/TBI prep with sirolimus/MMF as GVHD prophylaxis is administered followed by an umbilical cord blood transplant on Day 0 per institutional guidelines

The Treg cell infusion is given no sooner than 1 hour, but within 24 hours after the cord blood infusion.

Begin G-CSF Day +5 continuing until ANC ≥ 2,500 for 2 consecutive days.

Supportive care will follow institutional guidelines

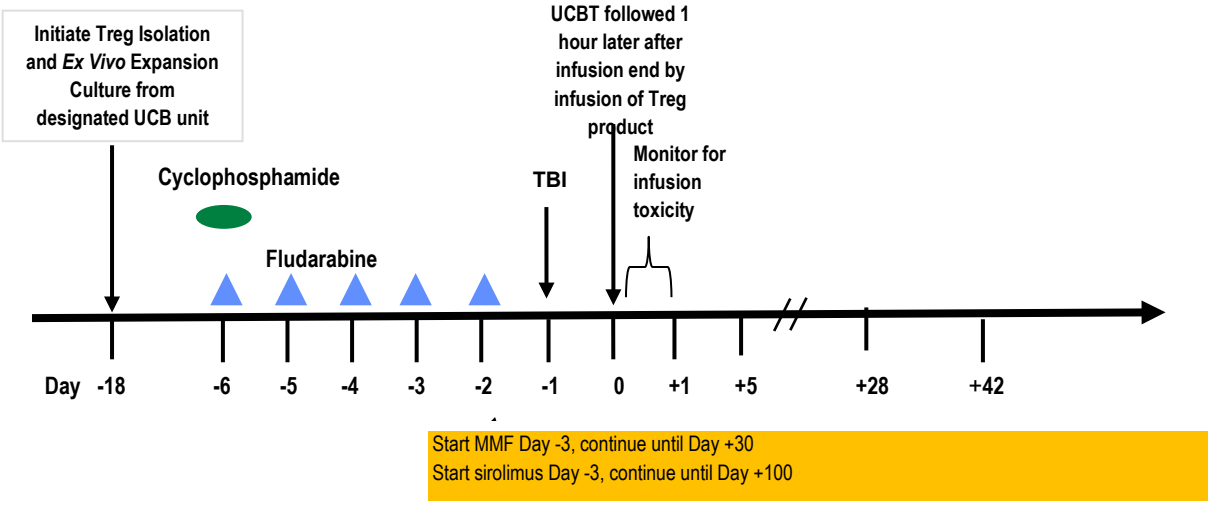
Enrollment: 10 patients will be enrolled over a period of 18 months

Study Schema

Identify cord blood units: 1 or 2 for the UCBT and an additional cord for culture

Treg product manufacturing at MCT:
Day -18: Initiate UCB Treg Isolation and *Ex Vivo* Expansion Culture from clinical grade research directed UCB unit
Day 0: Prepare final Treg cell product: Thaw of UCB graft unit(s) and enumerate CD3+ cells, calculate Treg cell dose at a 1:1 Treg:CD3+ cell ratio for final Treg cell product

Admit patient to hospital and begin allopurinol evening of day -7



Cyclophosphamide 50 mg/kg IV over 2 hours on Day -6

Fludarabine 30mg/m^2 IV over 1 hour on Day -6, -5, -4, -3, and -2

TBI Total Body Irradiation 200 cGy as a single dose

DUCBT followed by Tregs –umbilical cord blood transplant (FIRST) followed by the Treg cell infusion (SECOND) no sooner than 1 hour, but within 24 hours after the cord blood infusion.

Note: The 1st 3 patients enrolled in this study received ultra-low dose IL-2 post cell infusion; however due unacceptable toxicity, IL-2 was eliminated from the treatment plan effective with the January 2018 protocol version.

1 Objectives

1.1 Primary Objective

To obtain preliminary estimate of efficacy as measured by the length of Treg survival after infusion of Treg

1.2 Secondary Objectives

To obtain preliminary estimates of safety as measured by:

- Probability of grade II-IV aGVHD
- Probability of grade III-IV aGVHD
- Probability of treatment related mortality (TRM) at 6 months
- Probability of relapse at 1 year
- Probability of viral and fungal infections by 1 year
- Pattern of immune reconstitution
- The proportion of patients with detectable Treg cells at Day 14 post infusion
- Toxicity

1.3 Transplant Related Objectives

- Determine the incidence of chimerism at Day +100
- Determine the probability of survival at 1 year
- Determine the incidence of neutrophil recovery at Day 42
- Determine the incidence of platelet recovery at 1 year
- Determine the incidence of chronic GVHD at 1 year

2 Background

2.1 Adoptive Transfer of UCB-derived Regulatory T Cells

Regulatory T cells (Treg) represent a novel cell-based approach for potentially reducing the risk of severe acute graft-versus-host disease (aGVHD). Treg are a subset of CD4+ T cells that co-express CD25 (IL-2Rα chain) and high levels of Foxp3 (1) and are dependent on IL-2 (2). Our group and others have shown that in murine models lethal acute GVHD can be prevented by Treg with enhanced survival (3-8). We tested different Treg:T-effector cell ratios and observed moderate suppression at a 1:1 ratio, but almost complete suppression of GVHD at a 3:1 ratio. Further, in these models of acute GVHD CD4+/CD25+ Treg cells functioned at least in part through the suppression of CD8+ effector cells expansion in GVHD target organs (7). In contrast, depletion of CD4+/CD25+ Treg cells increased aGVHD lethality (5). Further, Treg inhibited the development of chronic GVHD (9-11) and

facilitated engraftment in murine models of allogeneic transplantation (7, 12, 13).

Double umbilical cord blood transplantation (DUCBT) has been shown to overcome the cell dose limitation that often prevents the use of this treatment modality in adults and larger adolescents (14-16). However, compared to single UCBT, a significantly higher risk of grade 2 aGVHD (17) is observed after DUCBT. Regardless of the source of allogeneic hematopoietic stem cells (HSC), severe forms of aGVHD are associated with an increased risk of morbidity and mortality (15, 17).

We conducted a phase I dose escalation trial to study the safety and feasibility of the infusion of Treg isolated from a partially HLA-matched UCB unit and ex vivo expanded in culture (18). The dose levels tested were 1, 3, 10 or 30 x 10^5 Treg/kg actual body weight on day +1 with an additional cohort who received a second dose of 30 x 10^5 Treg/kg on day +15 after UCBT. The cell dose levels were planned based on the murine studies and on what we estimated we would be able to achieve with the available manufacturing methodology. Since all our prior dUCBT experience incorporated CsA immunoprophylaxis, the MTD was first determined in patients concomitantly receiving CsA. As CsA has been shown to potentially interfere with optimal Treg function and survival (19-22), the last cohort received Treg at the maximal dose but in the presence of MMF and sirolimus rather than CsA.

2.2 Treg Manufacture

In the dose escalation study, CD25+ Treg enriched cells were isolated from a partially HLA matched third UCB unit that was 4-6/6 HLA matched to the patient. As the Treg unit was not expected to persist long-term, no inter-unit matching was required between the Treg donor unit and the two HSC graft units. Enrichment of CD25+ cells was accomplished by positive selection with directly conjugated anti-CD25 magnetic microbeads (Miltenyi Biotec, Bergish Gladbach, Germany) and the CliniMACS device (23). Isolated cells were subsequently cultured with anti-CD3/anti-CD28 monoclonal antibody (mAb)-coated Dynabeads (Provided under Food and Drug Administration Investigational New Drug # 6675 by Dr. Carl June, University of Pennsylvania) at a 3:1 bead to cell ratio for 18 ± 1 days. On day 3, cultures were supplemented with 300 IU/ml IL-2 (Proleukin, Chiron Corporation, Emeryville, CA). All products passed lot release criteria that included: 7AAD viability ≥70%, CD4+CD25+ purity ≥60%, less than 10% CD4-/CD8+ cells, anti-CD3/anti-CD28 mAB bead count <100 per 3x10^6 cells, negative gram stain, and absence of endotoxin (≤5 EU/kg). Remaining cells were frozen

using a standard cryopreservative cocktail with Plasma-Lyte A (Baxter Healthcare Corp, Deerfield, IL), dimethylsulfoxide (final concentration 10%), and human serum albumin and stored in Cryocyte bags (Baxter Healthcare Corp, Deerfiled, IL), for the planned second infusion on day +15. For the 14 patients receiving a second infusion at day +15, cells were thawed, diluted with 5% albumin /10% Dextran 40 and underwent limited additional lot release testing that included acceptable pre-freeze lot release and post-thaw acridine orange/propidium iodine (AOPI) viability $\geq$ 50%; all met lot release criteria. In six products the expansion failed to achieve the target dose level requiring the patients to be treated at a lower dose level.

In this study we were able to achieve the highest target cell dose level but in the absence of dose limiting toxicities, the maximum tolerated dose was not reached. Thus, we amended the protocol in order to be able to test higher cell doses. Based on pre-clinical data, we modified the manufacturing methodology by introducing an anti-CD3/anti-CD28 mAB bead re-stimulation on day 12 of the UCB-derived Treg expansion culture period. Clinical validation runs suggested we would be able to achieve Treg cell doses in order 100 $x10^5$/kg. However, only 1 of 4 clinical products achieved the target cell dose.

We modified ex vivo UCB-derived Treg expansion methodology by substituting anti-CD3/anti-CD28 mAB bead stimulation by genetically modified K562 cell line named KT64/86. This cell line expresses the CD64, the high affinity receptor for the Fc portion of antibodies loaded anti-CD3 and the CD86, the natural ligand of CD28. This allowed us to achieve 10-100–fold greater expansion, of a Treg product that is as suppressive and with lower proportion of IL-2-producing T-cells (non-Treg cells) as compared to the bead-based manufacture methodology. This methodology consistently produced Tregs in doses up to 100 x 10^6/kg without dose limiting toxicity making us confident that a Treg:CD3+ ratio up to 7:1 was achievable in this clinical trial.

2.3 Clinical Results with Bead Expanded Tregs

Twenty-three patients, median age of 52 years (r, 24-68yrs) and median weight 77kg (r, 50-133 kg), were treated between 9/2007 and 10/2009. The median expansion was 211-fold (range, 13-1796). Prior to and after expansion, the median proportion of CD4+/CD25+ cells was 65% (range, 11-87%) upon selection and 86% (range, 62-97%) and the end of the culture period. All Treg products were suppressive in vitro with median suppression at the end of the culture period of 86% (r, 39-95%) at a 1:4 ratio with an

inverse correlation between post-expansion absolute number of CD4+/CD25+ cells and the level of suppression (R=-0.48, p=.03). In contrast, there was no correlation between the absolute number or proportion of CD4+/CD25+ cells pre-expansion and proportion of CD4+/CD25+ cells post-culture, fold expansion and degree of suppression.

Seventeen of 23 (74%) received the Treg infusion at the target cell dose level. Five patients received a Treg dose less than the prescribed cell dose due to insufficient culture expansion (1 received a lower than prescribed dose because of peri-transplant morbidities prior to infusion on day +15). Of the 14 patients scheduled to receive two doses, 13 received the targeted dose of 30 x 10^5/kg on days +1 and +15 and 1 patient receive 2 doses of 21 x 10^5/kg (less than the planned dose due to insufficient culture expansion). Overall, 18 patients received a total Treg cell dose ≥30 x 10^5/kg.

2.4 Maximum Tolerated Dose: Treg Infusional Toxicity Profile and Detection Kinetics of Bead Expanded Tregs

No dose limiting toxicity was observed. There were 2 patients with three Grade 3 hypertension, one following infusion of a fresh and two following infusion of cryopreserved Treg product, with all resolving with standard clinical management. Two patients had Grade 2 neurological changes prior to the infusion that were attributed to previously prescribed narcotic medication.

In the 23 patients studied, the median proportion of peripheral CD4+ T cells that were FoxP3+CD127- was 27% (range, 1-78%) at 4 hours post infusion (T4), 30% (range, 8-77%) on day +2, 27% (range, 5-72%) on day +4, 21% (range, 7-83%) on day +7, and 10.% (range, 1-36%) on day +14. Notably, among the 18 patients who received ≥ 30 x10^5/kg Treg, the median proportion of peripheral blood CD4+/FoxP3+/CD127- was not statistically different for all time points in recipients of CsA (n=12,) and sirolimus (n=6,). Specifically, the proportion of CD4+/FoxP3+/CD127- cells in recipients of CsA vs. sirolimus was 27% (range, 1.-78%) vs. 43% (range, 27-46%) at T4, 35% (range, 10-77%) vs. 41% (range,30-54%) on day +2, 35% (range, 5-71%) vs. 49% (range, 27-54%) on day +4, 27% (range, 13-83%) vs. 34% (range, 17-47%) on day +7, and 12% (range, 1-36%) vs. 13% (range, 9-29%) on day +14 after UCB-transplantation. The absolute number of CD4+/FoxP3+/CD127- cells in the peripheral blood of the cryopreserved product infused on day +15 (CsA n=8; sirolimus n=6) was lower compared to freshly infused Treg on day +1.

As expected, Treg circulating after transplant were derived from the patient and HSC UCB graft as well as the manufactured Treg product. Based on

informative HLA-marker on the UCB-Treg unit we were able to track the ex vivo expanded Treg after infusion in 7 patients. The absolute number of product derived Treg in the peripheral blood for all patients peaked on day +2 at 30% (range, 8-77%), and for those who received ≥ 30 x 10^5/kg at day +4 representing up to 40% (range, 5-72%) of all Treg in the circulation. UCB-derived Treg were detected for up to 14 days with no difference in the kinetics in recipients of CsA vs. sirolimus.

2.5 Engraftment with Bead Expanded Tregs

Sustained donor-derived neutrophil engraftment was observed in 87% (95%CI, 70-97%) in recipients of Treg and 89% (95%CI, 70-98%) for those patients who received ≥ 30 x10^5/kg. Of the 2 patients who had graft failure both had HHV-6 viremia. One patient recovered counts at day +51 with donor-derived hematopoiesis without further intervention; the second patient received a second UCBT at day +60. The incidence of platelet recovery by day +100 was 74% (95%CI, 51-97) at a median of 46 days (range, 27-87 days) and for those patients who received ≥ 30 x10^5/kg was 78% (95%CI, 53-100%) at a median of 43 days (range, 29-83). Neither sustained donor engraftment nor platelet recovery was adversely affected by Tregs as compared to historical controls where recoveries were 86% (95%CI, 79-92%) and 67% (95%CI, 56-78%), respectively**.** The median marrow chimerism at day +21 was 91% (r, 37-100%) with 12 of 21 (57%) engrafted patients having dual chimerism. The prevalence of dual chimerism after Treg tended to be higher than that observed in historical controls which was 36% (p=.06). By day + 100, 2 patients (11%), both receiving 30 x10^5/kg Treg on days +1 and +15 with CsA/MMF immunosuppression, had persistent dual chimerism which is similar to that observed in historical controls (11%, p=.68). While immunological properties of infused UCB Treg may be promoting early dual chimerism, the pattern of long-term single donor chimerism is unchanged.

2.6 Graft-Versus-Host Disease (GVHD) with Bead Expanded Tregs

The incidence of grades II-IV aGVHD for all patients was 43% (95%CI, 23-64%) at a median time of 38 days (range, 24-86), and was similar in those who received a total Treg dose ≥ 30 x10^5/kg (39% [95%CI, 16-61%) at a median of 39 days [range, 29-86]). This was lower than that observed in historical controls (61% [95%CI, 51-72%] at a median of 30 days [range, 14-73). The incidence of grades III-IV acute GVHD for all patients was 17% (95%CI, 2-23%) at a median time of 51.5 days (range, 24-86), and for those who received a Treg dose ≥ 30 x10^5/kg, it was 11% (95%CI, 0-25) at a median time of 76 days (range, 66-86). This was similar to that observed in historical controls (23% [95%CI, 15-31%] at a median of 29 days [range, 14-72]). Two of 14 (14 %) patients at risk developed chronic GVHD. Thus far,

chronic GVHD has not been observed among patients who received a Treg dose ≥30 x10^5/kg. These results compare favorably with historical controls where the incidence of chronic GVHD was 26% (95%CI, 17-35%). Taken together these data support further investigation to determine whether ex vivo expanded and activated UCB Treg can suppress GVHD.

2.7 Opportunistic Infections with Bead Expanded Tregs

While nTregs are potentially immune suppressive, in our initial report we did not observe an increase in the cumulative incidence of opportunistic infections (OI) (24). However, there was still the possibility that individual patients were having a greater number of recurrent infections. Thus, we revisited the risk of infection question by estimating the cumulative density of infections in nTreg recipients and historical controls. The incidence of OI at day +180 was 58% (95%CI, 38-79%) in nTreg recipients and 60% (95%CI, 46-74%) historical controls (p=0.89), at a median of 28 (9-87) and 41 (1-110) days after UCB transplantation, respectively. Viral infections represented the majority of OI observed for nTreg (58% [95%CI, 38-79%]) and historical control (55% [95%CI, 42-69%]) patient populations (p=.67). The overall day 0 to day +180 cumulative density of OI was 2.10 infections/1000 patient-days in nTreg and 1.52 infections/1000 patient-days in non-Treg patients (RR 1.39, p=.08). However, in our initial report we showed that adoptively transferred nTregs were present in the peripheral blood of patients up to 14 days after the infusion of fresh and up to 4 days after the infusion of cryopreserved product (18). Notably, between day zero and day +30 post-UCB transplantation there was significantly higher cumulative density of OI in Treg (18.06 infections/1000 patient-days) as compared to historical controls (7.71 infections/1000 patient-days) patients (univariate RR 5.35, p=.02). In contrast, there was no statistical difference in the cumulative incidence of OIs from day +31 to day +180 post-UCB transplantation, with the density of infection in nTreg of 4.22/1000 patient-days versus 7.22/1000 patient-days in historical controls (univariate RR 0.58, p=.07) when nTregs were no longer detectable in the peripheral blood of patients. However, as we are comparing patients in a phase I study, who are very closely monitored, to historical controls, it is possible that our findings were the result of observation bias. Close monitoring of opportunist infection will remain part of Treg recipient's clinical care.

2.8 Relapse, Non-Relapse Mortality and Disease-Free Survival with Bead Expanded Tregs

At a minimum follow-up of 2 years for all patients, long-term outcomes remain unaffected by nTreg infusion. The median follow up of survivors is 2.6 years (range, 2.0-3.3) for nTreg and 4.8 years (range, 2.0-6.9) for historical control patients. The cumulative incidence of relapse at 2 years was 45% (95%CI, 23-67%) for nTreg and 52% (95%CI, 37-66) for historical controls (p=.58). The cumulative incidence of non-relapse mortality at 2 years was 45% (95%CI, 23-67%) for nTreg and 52% (95%CI, 38-66%) for historical controls (p=.62). The DFS at 2 years was 33% (95%CI, 16-52%) for nTreg and 34% (95%CI, 46%) for historical controls (p=.95).

2.9 Results of KT64/86 Expanded Tregs

Thirteen Treg products were manufactured and eleven were infused. Five products did not reach the target dose level, and 2 of these 5 products failed to reach the minimum required dose of 3 x 10^6/kg for infusion. Among the 11 infused products the median number of nucleated cells was 40 billion (range (r), 2-147 billion; inter-quartile range, 39-44 billion) corresponding to a median TNC expansion of 13,000-fold (r, 1,352-27,183 fold), of which the median proportion of CD4+CD25+ cells was 97% (r, 88-99%) and with 87% (r, 78-95%) expressing CD4+FoxP3+CD127-. In 6 evaluable products, the median in vitro suppressor function of CD3+ bead stimulated T cells at a 1:4 T effector to Treg ratio was 53% (range, 45-71%).

Infusional toxicities were monitored prior to the Treg product infusion, and then at 24 and 48h post-infusion. Toxicities were mild and no dose limiting toxicity was observed in doses up to 100 x 10^6/kg.

An informative HLA-marker (unique to the Treg donor unit) was available in 7 of 11 patients. The presence of the Treg product in the peripheral blood was tracked by flow cytometry with progressively greater detection correlating with higher infused Treg cell doses. Despite a higher peak, greater infused cell doses did not yield longer persistence of peripheral blood Tregs; none persisted after 14 days.

Acute Graft-versus-Host Disease

Among 11 recipients of 3-100 x 10^6 Treg/kg, the cumulative incidence of grade II-IV acute GVHD at 100 days was 9% (95%CI, 0-25%). One patient developed a limited skin rash (50% of body surface) on day +16 with a skin biopsy not diagnostic for acute GVHD. The patient was treated with steroids with a clinical differential diagnosis of either engraftment syndrome or early

onset grade II acute GVHD. Steroids were tapered rapidly over 4 weeks without recurrence of rash or development of other possible GVHD symptoms. One other patient developed biopsy-proven grade III gastrointestinal acute GVHD on day +119, responded promptly to systemic steroids and was tapered off all immune suppression within 6 months. In contrast, in the contemporary control group, that consisted of patients who received same conditioning regimen and immune suppression but no Tregs, 10 of 22 patients developed grades II-IV acute GVHD, with 4 cases of grade II and 6 cases of grade III-IV acute GVHD, for a cumulative incidence of grade II-IV GVHD of 45% (95%CI, 24-67%) (p=.05) and grade III-IV of 27% (95%CI, 9-46%) (p=.06). None of the Treg recipients and 3 (14%) patients in the control group developed chronic GVHD during follow-up to date.

Hematopoietic Recovery

The cumulative incidence of neutrophil recovery by day +42 was 91% (95%CI, 67-99%) in Treg recipients as compared to 86% (95%CI, 69-97%) in controls. There was 1 primary graft failure in the 11 Treg recipients versus 3 in 22 controls. The cumulative incidence of platelet recovery > 20,000/μL by day +180 was 82% (95%CI, 55-100%) for Treg recipients and 82% (95%CI, 59-100%) for controls. The median combined unit bone marrow chimerism at day +21 was 90% (r, 66-100%) for Treg recipients and 90% (95%CI, 19-100%) (p=.99) for controls, and at day +100 was 100% (r, 100-100) for Treg recipients and 100% (r, 97-100%) for controls (p=.77).

Infections and Immune Reconstitution

In the Treg group there were a total of 17 infection events before day +100 in 9 patients versus 31 in 14 of the Siro/MMF controls. The density of bacterial, viral and fungal infections (infections per 1000 patient days) was similar in the two groups. There were 2 CMV and 9 HHV-6 reactivations in Treg recipients compared to 1 CMV and 8 HHV-6 reactivations in controls. Out to day +180, there was faster recovery of the absolute number of CD4+ and a subset of naïve CD4+CD45RA+CCR7+ lymphocytes in Treg recipients as compared to controls. In contrast, the recovery of other CD4+ subsets and CD8+ and its subsets, CD19+ and CD56+ cells were similar in the two groups.

Relapse, Non-Relapse Mortality, Survival and Disease-Free Survival

Malignant relapse has occurred in only 3 of 9 surviving patients treated with Treg and 8 of 20 in the control group. At 6 months, 2 Treg patients and 2 controls died of non-relapse causes resulting in similar cumulative incidence

of NRM. Disease-free survival at 1 year is 55% (95%CI, 23-78%) among Treg recipients and 55% (95%CI, 32-72%) in the control group (p=.76). The estimated overall survival at 1 year was 81% (95%CI, 42-95%) among Treg recipients and 61% (95%CI, 37-79%) in the control group (p=.30). The causes of death in the Treg group were graft rejection and acute respiratory distress syndrome (1 each), while in the control group causes of death were graft rejection (n=1), acute GVHD (n=2), and relapse (n=5).

In summary, we 1) demonstrated the ability to consistently manufacture Treg doses up to 100 x10e6/kg which allows for Treg:Teffector ratio of up to 7:1, and 2) observed a remarkably low risk of acute GVHD. This promising result is the first clear demonstration that ex vivo expanded UCB Treg are potent suppressors of acute GVHD in humans and sets the stage for a future pivotal clinical trial that will assess the clinical impact of Treg GVHD prophylaxis on immune recovery and risk of leukemia relapse.

2.10 Ultra-Low IL-2 for GVHD Prophylaxis (removed from the treatment plan with the January 2018 protocol version)

As described above, Tregs do not produce but are dependent on IL-2 for survival and proliferation. After studying the administration of ultra-low dose (ULD) IL-2 in normal volunteers (25, 26), researchers at Baylor conducted a study administering ULD IL-2 to patients undergoing allogeneic transplantation from matched sibling and unrelated donors (25, 26). The IL-2 was started within 30 days of transplantation and administered at 100,000-200,000 IU/m2, subcutaneously (SQ), thrice weekly for 6 to 12 weeks. They demonstrated a very low incidence of GVHD with 3 of 16 patients developing grade I GVHD of the skin. The also observed a significant increase in the proportion of circulating Tregs from 4.8% to 11.1%. Notably, the increase on the proportion of circulating Tregs was observed after just 1 week of IL-2 administration. Adverse effects after IL-2 administration were all grade 1 and included muscle aches, arthralgias, fatigue, nausea, and decreased appetite. While sample was limited, there was no excess risk of GVHD, infections or relapse.

After treating two patients on this protocol with ULD-IL2 we observed fevers, chills, fluid, retention, creatinine elevation, and lung infiltrates. One of these patients also had transient mental status changes. While both cases had multiple confounding factors we could not rule out that the observed toxicity was at least in part related to ULD-IL2. In addition, this early post-transplantation time when fevers, infections and several of these side effects are common, the addition of ULD-IL2 added complexity to patient

management. Thus, we decided to discontinue the administration of ULD-IL2 with the January 2018 protocol revision and continue this study in our UCB Treg adoptive transfer, which we demonstrated in the previous study to be safe and well tolerated.

3 Summary and Rationale

Our preclinical studies demonstrated a marked reduction in GVHD at Treg:T effector ratios of 1:1 and 3:1 (7), necessitating infusion of Treg doses of >15 x 10^6/kg to achieve that ratio for the median infused CD3 cell dose in double UCB grafts. In our KT64/86 expanded Tregs we achieved Treg:Teffector ratio of up to 7:1. Despite the relatively high dose of adoptively transferred Tregs, they become undetectable in the patient's blood by 14 days post-infusion.

As our ultimate goal is to design a strategy that will a) significantly reduce the risk of GVHD, b) eliminate the requirement of prolonged immune suppressive pharmacological therapy, and c) enhance T cell immune reconstitution. In order to progress towards this goal, we propose this pilot study to provide preliminary estimates of efficacy, safety and to show the feasibility after combining a fixed Treg:CD3+ cells ratio (1:1).

This pilot study will help in designing future larger scale studies to hopefully provide evidence on improved survival of adoptively transferred Tregs. This could have implications on the effectiveness of Treg in preventing GVHD and establish a new platform for evaluating new methods for further enhancing T cell recovery and reducing the risk of opportunistic infection—the single greatest barrier to successful allogeneic HSC transplant.

4 Study Design

This is single center pilot study of a non-myeloablative umbilical cord blood transplant for the treatment of a hematological malignancy with a single infusion of T regulatory (Treg) given shortly after UCB transplantation.

The nTreg cells are manufactured from a cord blood unit that is not part of the graft for infusion on Day 0 at least 1 hour after the UCB transplant. The CD3+ cell content from the graft UCB unit(s) is enumerated upon thaw (Day 0) from which the Treg cell dose for the final product is calculated using assigned 1:1 Treg:CD3+ cell ratio.

If any of the cord blood unit(s) used for the graft are unlicensed, the participant will co-enroll on University of Minnesota protocol MT2011-13R "Infusion of Cell Populations from Unlicensed Umbilical Cord Blood Units" as the unit(s) used for the transplant will be minimally manipulated.

5 Patient Selection

Study entry is open to adult patients regardless of gender or ethnic background. While there will be every effort to seek out and include women and minority patients, the patient population is expected to be no different than that of other high risk malignancies transplant studies at the University Of Minnesota.

5.1 Age, Graft Cell Dose and Graft HLA Criteria and Treg Unit

- Must be ≥18, but < 70 years of age with no matched 7/8 or 8/8 sibling donor - patients ≥ 70 and ≤ 75 years of age may be eligible if they have a Co-Morbidity score ≤ 2 (http://www.qxmd.com/calculate-online/hematology/hct-ci)
- UCB unit(s) composing the graft will be selected according to the current University of Minnesota umbilical cord blood graft selection algorithm plus an additional cord blood unit to be used as the source to manufacture the Treg product. This UCB unit must be matched at 4-6/6 to the patient, considering HLA-A, B at the antigen level and DRB1 at the allele level.

5.2 Disease Criteria

Acute Leukemias: Must be in remission by morphology (around 5% blasts but < 20% blasts by morphology). Also a small percentage of blasts that is equivocal between marrow regeneration versus early relapse are acceptable provided there are no associated cytogenetic markers consistent with relapse.

- Acute Lymphoblastic Leukemia (ALL) second or greater CR; CR1 unable to tolerate consolidation chemotherapy due to chemotherapy-related toxicities; CR1 high-risk ALL.

  High risk ALL is defined as having one of the following:

  - Evidence of high risk cytogenetics such as t(9;22), t(1;19), t(4;11), other MLL rearrangements, *IKZF1*
  - 30 years of age or older at diagnosis
  - White blood cell counts of greater than 30,000/mcL (B-ALL) or greater than 100,000/mcL (T-ALL) at diagnosis
  - CNS leukemia involvement during the course of disease

  - Slow cytologic response (>10% lymphoblasts in bone marrow on Day 14 of induction therapy)
  - Evidence of persistent immonophenotypic or molecular minimal residual disease (MRD) at the end of induction and consolidation therapy

- Acute Myelogenous Leukemia (AML) and related precursor neoplasms: 2nd or greater complete remission (CR); first complete remission (CR1) in patients > 60 years old; CR1 in ≤ 60 years old that is NOT considered as favorable-risk.

  Favorable risk is defined as having one of the following:

  - t(8,21) without *cKIT* mutation
  - inv(16) or t(16;16) without *cKIT* mutation
  - Normal karyotype with mutated NPM1 and wild type FLT-ITD
  - Normal karyotype with double mutated *CEBPA*
  - Acute prolymphocytic leukemia (APL) in first molecular remission at end of consolidation

- Biphenotypic/Undifferentiated/Prolymphocytic Leukemias in first or subsequent CR, adult T-cell leukemia/lymphoma in first or subsequent CR

Burkitt's Lymphoma in CR2 or subsequent CR

Natural Killer Cell Malignancies

Chronic Myelogenous Leukemia: all types except refractory blast crisis. Chronic phase patients must have failed at least two different tyrosine-kinase inhibitors (TKIs), or been intolerant to all available TKIs or have *T315I* mutation.

Myelodysplastic Syndrome: IPSS INT-2 or High Risk; R-IPSS High or Very High; WHO classification: RAEB-1, RAEB-2; Severe Cytopenias: ANC < 0.8, Anemia or thrombocytopenia requiring transfusion; Poor or very poor risk cytogenetics based on IPSS or R-IPSS definitions; therapy-related MDS. Blasts must be < 5% by bone marrow aspirate morphology. If ≥5% blasts, patient requires chemotherapy for cytoreduction to <5% blasts prior to transplantation.

Large-Cell Lymphoma, Hodgkin Lymphoma and Multiple Myeloma with chemotherapy sensitive disease that has failed or patients who are ineligible for an autologous transplant.

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL), Marginal Zone B-Cell Lymphoma, Follicular Lymphoma, which have progressed within 12 months of achieving a partial or complete remission. Patients who had remissions lasting > 12 months are eligible after at least two prior therapies. Patients with bulky disease should be considered for debulking chemotherapy before transplant. Patients with refractory disease are eligible, unless has bulky disease and an estimated tumor doubling time of less than one month.

Lymphoplasmacytic Lymphoma, Mantle-Cell Lymphoma, Prolymphocytic Leukemia are eligible after initial therapy if chemotherapy sensitive.

Patients must have undergone an autologous transplant ≤ 12 months prior to transplant on this study or have received multi-agent or immunosuppressive chemotherapy within 3 months of the preparative regimen.

5.3 Performance Status, Organ Function, Contraception Use

- Karnofsky score ≥ 70% (Appendix II)
- Adequate organ function within 14 days (30 days for cardiac and pulmonary) of registration on-study defined as:
  - Renal: creatinine ≤ 2.0 mg/dL, for patient with a creatinine > 1.2 mg/dL or a history of renal dysfunction an estimated glomerular filtration rate ≥ 40 mL/min/1.73 m^2 is required
  - ALT, AST and alkaline phosphatase ≤ 5 x upper limit of normal and total bilirubin ≤ 2.5 mg/dL except for patients with Gilbert's syndrome or hemolysis
  - Pulmonary function: DLCO, FEV_1, FVC ≥ 40% predicted, and absence of O_2 requirements.
  - Cardiac: Absence of decompensated congestive heart failure, or uncontrolled arrhythmia and left ventricular ejection fraction ≥ 40%.
- Sexually active females of childbearing potential and males with partners of child-bearing potential must agree to use adequate birth control during study treatment.
- Voluntary written consent

5.4 Exclusions

- Untreated active infection
- History of HIV infection
- Pregnant or breast feeding. The agents used in this study may be teratogenic to a fetus and there is no information on the excretion of agents

into breast milk. Females of childbearing potential must have a blood test or urine study within 14 days prior to registration to rule out pregnancy
- Prior allogeneic transplantation
- Less than 3 months from myeloablative conditioning for autologous transplantation (if applicable)
- Evidence of progressive disease by imaging modalities or biopsy - persistent PET activity, though possibly related to lymphoma, is not an exclusion criterion in the absence of CT changes indicating progression.
- CML in blast crisis
- Large cell lymphoma, mantle cell lymphoma and Hodgkin disease that is progressing on salvage therapy
- Active central nervous system malignancy

6 Patient Registration and Study Enrollment

Written consent must be obtained prior to the performance of any research related tests or procedures. Consent is usually obtained before final eligibility is determined.

6.1 Registration with the Masonic Cancer Center Clinical Trials Office

Any patient who has been consented is to be registered in OnCore by the Primary Clinical Research Coordinator (PCRC) or designee. If a patient is consented, but not enrolled, the patient's record is updated in OnCore as a screen failure and reason for exclusion recorded.

6.2 Study Enrollment with the Masonic Cancer Center Clinical Trials Office

To be eligible for study enrollment, the patient must sign the treatment consent and meet each of the inclusion criteria and none of the exclusion on the eligibility checklist (Appendix I) based on the eligibility assessment documented in the patient's medical record.

The Primary Clinical Research Coordinator (PCRC) or designee will assign the study treatment arm and add the on-treatment date to complete enrollment.

6.3 Patients Who Do Not Begin Study Treatment

If a patient is enrolled to the study, and is later found not able to the preparative regimen, for whatever reason, the patient will be considered a screen failure and treated (transplanted) at the physician's discretion. The Primary Clinical Research Coordinator or designee will update OnCore of the patient's non-treatment status and notify the Principal Investigator. Study data

will be collected until the time the patient is taken off study. The patient will be replaced as needed to fulfill enrollment requirements.

7 Treatment Plan

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, methylprednisone, G-CSF, etc.), and will not be considered a protocol deviation.

All patients will receive the non-myeloablative conditioning regimen below:

Treatment Day	Treatment	Protocol Section
Day -7	Begin allopurinol (Day -7 to day 0)	
Day -6	Fludarabine 30 mg/m^2 IV over 1 hour Cyclophosphamide 50 mg/kg IV over 2 hours Antiemetics and fluid flush per institutional guidelines	7.1
Day -5	Fludarabine 30 mg/m^2 IV over 1 hour	7.1
Day -4	Fludarabine 30 mg/m^2 IV over 1 hour	7.1
Day -3	Fludarabine 30 mg/m^2 IV over 1 hour Begin MMF and Sirolimus	7.1 7.2
Day -2	Fludarabine 30 mg/m^2 IV over 1 hour	7.1
Day -1	TBI 200 cGy	7.1
Day 0	Umbilical cord blood transplant (FIRST) followed by the Treg cell infusion (SECOND) no sooner than 1 hour, but within 24 hours after the end of the cord blood infusions	7.3 and 7.4
Day +5	Begin G-CSF day +5 continuing until ANC ≥ 2,500 for 2 consecutive days	7.5

7.1 Preparative Regimen (Day -6 to Day -1)

Administer **fludarabine and cyclophosphamide** per University Of Minnesota institutional guidelines for a non-myeloablative preparative regimen.

Fludarabine 30 mg/m^2 is administered as a 30-60 minute infusion per institutional guidelines on Day -6 through Day -2. Dose adjustments will be made for adult patients with renal impairment defined as CrCL < 70mL/minute. Fludarabine dose MAY also be reduced to this dose if there is

prior malignancy involvement of the central nervous system with intrathecal chemotherapy and/or cranio-spinal irradiation

Cyclophosphamide Hydration prior to cyclophosphamide will be given according to recommended institutional standards, starting 12 hours prior to cyclophosphamide.

Mesna dose will be 100% of the cyclophosphamide dose being given and divided into 5 doses. 20% of the total dose will be given prior to the start of cyclophosphamide, and then 3, 6, 9, and 12 hours after the start of cyclophosphamide.

Cyclophosphamide 50 mg/kg will be administered as a 2 hour intravenous infusion on Day –6. Cyclophosphamide dosing is calculated based on ABW (actual weight) unless ABW is >150% of the IBW (Ideal Body Weight). Then the dose should be computed using adjusted body weight.

Ideal body weight is calculated using 50kg + [2.3kg x(height in inches – 60)] for men; 45.5kg + [2.3kg x(height in inches – 60)] for women.

Adjusted body weight = IBW + 0.5(ABW-IBW).

Total Body Irradiation (TBI)

All patients who have had previous radiation therapy or TBI will be seen by Radiation Oncology prior to entrance on the protocol for approval for additional 200 cGy of TBI. TBI may be delivered by local guidelines provided the effective dose is equivalent to what is recommended in the TBI Guidelines.

Patients ineligible for this protocol include those who have had previous irradiation to areas of the body such that the Radiation Oncologist feels that even a relatively small dose of total body irradiation (TBI) cannot safely be given.

Total Body Irradiation 200 cGy administered on Day -1 in a single fraction will be given at a dose rate of 10-19 cGy/minute prescribed to the midplane of the patient at the level of the umbilicus.

The TBI will be delivered with right and left lateral fields with the patient semi-recumbent in a semi-fetal position with their arms at their sides.

Based on measurement of transverse thickness, aluminum compensators will be used to ensure that the dose homogeneity across the fields is within 10% of the prescribed dose. Usually head/ neck, leg and lung compensators are

used (although based on calculated mid-mediastinal doses, lung compensators are often not needed if the thickness of the arms, which partially shield the lung, are taken into the thickness consideration).

TBI will be delivered with a linear accelerator using 6, 10, or 18 MV photons. The energy used will be based on the calculated dose to midline at points along the patient's torso. The lowest energy that gives 90-100% of the prescriptions point dose will be used.

A beam "spoiler" will be used to ensure a full skin dose.

Half value layer lung and kidney blocks will not be utilized for patients who have not previously received total body irradiation.

Refer to appendix III for side effects related to the preparative regimen.

7.2 GVHD Prophylaxis (start Day -3)

Immunosuppression will consist of sirolimus and mycophenolate mofetil (MMF).

7.2.1 Sirolimus

Sirolimus will be administered starting at Day -3 with 8mg-12mg oral loading dose followed by single dose 4 mg/day with a target serum concentration of 3 to 12 mg/mL by high-performance liquid chromatography (HPLC). Levels are to be monitored 3 times/week in the first week, weekly until Day +60, and as clinically indicated until Day +100 post-transplantation. In the absence of acute GVHD sirolimus may be tapered starting at Day +100 and eliminated by Day +180 post-transplantation.

7.2.2 Mycophenolate Mofetil (MMF)

Mycophenolate mofetil (MMF) 3 gram/day IV/PO divided in 2 or 3 doses. MMF dosing will be monitored and altered as clinically appropriate based on institutional guidelines. Patients will be eligible for MMF dosing and pharmacokinetics studies.

Stop MMF at Day +30 *or* 7 days after neutrophil recovery, whichever day is later, if no acute GVHD. (Definition of engraftment is 1st day of 2 consecutive days of absolute neutrophil count [ANC) ≥ 0.5 x 10^9 /L]).

If at Day 28 the WBC is < 200 and marrow cellularity is < 5%, but there is donor chimerism, MMF should be discontinued and institutional guidelines for the management of delayed neutrophil recovery should

be followed. In the absence of improvement a second transplant may be considered as clinically indicated.

If the patient has acute GVHD requiring systemic therapy, MMF may be stopped 7 days after initiation of systemic therapy for acute GVHD (e.g. resolution of skin rash, vomiting, and diarrhea).

7.3 UCB Infusion (Day 0)

There will be a minimum interval of 1 hour between the infusion of the UCB grafts (FIRST) and the Treg cell product (SECOND).

The pre-medication and hydration regimen will be given following University of Minnesota institutional guidelines. No planned steroids are to be given in the 24 hours prior to the Treg infusion.

The graft UCB units will be administrated by IV infusion without in-line filtration per University of Minnesota institutional guidelines. The infusion of the first UCB unit should begin within 15 minutes, and no later than 30 minutes after arrival on the Unit. Both cords will be infused within 30-60 minutes of each other as deemed clinically safe by the BMT attending or designee.

Vital signs will be checked before and every 15 minutes during the infusions, at the completion of each infusion, and one hour post infusion the 2^{nd} unit infusion prior to the Treg infusion.

7.4 Infusion of the Treg Cells (Day 0 after UCBT)

7.4.1 Product Final Preparation at MCT

On day 0, the dose of UCB derived Treg cells will be determined by the enumeration of the CD3+ cell from the UCB graft unit(s). A 1:1 Treg:CD3+ cell ratio will be calculated.

The UCB Treg cells may be infused fresh out of culture or cryopreserved in case the patient requires a delay in the transplant procedure for clinical reasons.

Leftover/un-infused cells may be stored frozen for future research.

Failure to Achieve Target Ratio Dose

If the product fails to achieve the planned 1:1 Treg:CD3+ cell ratio, the product may still be infused as long as there are at least ≥3 x 10^6/kg. Patients who receive Tregs will be evaluable for the purposes of the study.

Lot Release Failure

Following FDA regulations, if lot release criteria are not achieved, as described in the CMC document, the patient does not receive the product. Lab staff will notify the Medical Director and the Sponsor/Investigator who will contact the patient physician's (if applicable) in a timely fashion, for appropriate clinical action. If appropriate, the Sponsor/Investigator or his designee may contact the FDA to request permission to infuse the cell product. In the case where the Treg cells cannot be administered, the patient will be replaced.

7.4.2 Pre-Infusion Medication and Hydration Regimen

The hydration and pre-medication regimen will be given following University of Minnesota institutional guidelines. Patients weighing less than 40 kg may have the dose adjusted.

NO planned steroids will be given in the 24 HOURS PRIOR to the Treg infusion.

7.4.3 Infusion of the Treg Cell Product

The Tregs will be infused IV without in-line filtration within 1 to 4 hours of formulation and at least 1 hour after the second UCB unit has been infused, but no later than 24 hours after. If the Treg cells cannot be infused within 24 hours, the cells may be frozen for administration as soon as clinically possible.

Potential infusion related toxicity include acute allergic reactions. Patients will be closely monitored for the occurrence of hypotension, dyspnea and angiodema during the infusion and immediately thereafter. Infusion of Treg cells will be stopped if severe acute allergic reactions occur (e.g. Grade 4 toxicity based on the NCI's CTCAE 4 except for fevers alone).

A health care professional must be present during the nTreg cell infusion and available for 1 hour afterwards.

Emergency drugs such as epinephrine, hydrocortisone, diphenhydramine and atropine in appropriate dosages and dilutions should be available and administered according to institutional guidelines. Oxygen with nasal prongs for standby use should be present in the room.

7.4.4 Treg Cell Infusion Assessments

Vital signs will be monitored before the infusion start and every 15 minutes through 2 hours after the completion of the infusion, hourly for 4 additional hours and then vitals per post-transplant unit guidelines.

Patients will be monitored for infusion related toxicity for 24 hours following administration of the Treg cell product. Infusion related toxicity is defined as any non-hematologic reaction within 24 hours of administration of the cell product that cannot be explained by other transplant related procedures.

7.5 Supportive Care

Patients will receive transfusions, infection prophylaxis, growth factor and nutritional support according to the current University of Minnesota support protocol guidelines.

Begin G-CSF Day +5. Continue until ANC ≥ 2,500 for 2 consecutive days.

7.6 Duration of Study Participation

The study follow-up period is completed 100 days post-transplant when patient are evaluable for safety and efficacy of adoptive transfer of UCB Treg at a 1:1 ratio with CD3+ cells.

Patients will be followed for 2 years post-transplant per the standard of care schedule in Section 9.1; however direct study participation (endpoints, research sample collection, etc.) will end at Day 100.

Follow-up after 2 years will be per the University of Minnesota standard hematopoietic stem cell transplantation protocol for long-term follow-up.

8 Expected Toxicities of Treg Cell Product

Although Treg cells helped the donor cells to take hold (engraft) and reduced GVHD in laboratory models, it is not known how Treg cells will work in humans. Studies in animal showed that Treg cells are not detectable after 4-6 weeks.

While no side effects were identified in the animal testing, possible risks include: allergic reaction, increased risk of infection, increased risk of graft-versus host disease (the opposite effect), and increased risk of relapse or disease recurrence.

9 Schedule of Patient Activities

Scheduled evaluations after screening and until engraftment may be performed +/-3 days from the targeted date; assessments performed after engraftment and through day 100 may be done +/-7 days of the targeted date. After day 100 assessments may be done +/- 30 days of the targeted date. In addition, targeted days may be altered as clinically appropriate.

9.1 Standard of Care

Activity	Pre-BMT Work-Up	Day 1 To Engraftment[1]	Follow-Up Days 42-100	Follow-Up (>Day 100 through Day 720)
Consent	X			
Baseline Assessment	X			
Medical History	X	daily	weekly	X (day 180, 360, 720)
Physical Exam	X	daily	weekly	X (day 180, 360, 720)
Karnofsky Performance Status	X		day 100	X (day 180, 360, 720)
GVHD Assessment		daily	weekly, day 100	X (day 180, 360)
CBC/diff/plt	X	daily	weekly	X (day 180, 360, 720)
PT/PTT	X			
Viral Screen	X			
Basic metabolic panel		daily (on days CMP not done)		
Comprehensive metabolic panel	X	2x/wk	weekly	X (day 180, 360, 720)
Testing for anti-HLA antibodies[3]	X			
Urinalysis	X			
eGFR for adults with creat > 1.2 or hx or renal dysfunction	X			
Pregnancy test for FOCBP	X			
BM Biopsy chimerism		BM (day 21)	BM (day 100)	BM (day 360, 720)
Blood chimerism	Patient and UCB	PB (day 21)	PB (day 60)	Day 180
PFT/DLCO	X			
MUGA or Echo	X			
Chest CT	X[2]			
Disease Evaluation	X	X(day 21 to 28)	X (day 100)	X (day 360, 720)

1 engraftment defined as absolute neutrophil count (ANC) ≥ 5 X 10^8/L for 3 consecutive days

2 Patients with a history of MDS or a history of 2 or more consecutive inductions/re-inductions to treat acute leukemia or CML blast crisis or prolonged neutropenia of at least 2 months immediately preceding transplant should have a chest CT without contrast to exclude occult fungal infection prior to transplant.

3 obtain as soon as possible once the patient is determined to be a candidate for UCB transplantation in order to guide unit selection

NOTE: In certain clinical circumstances (e.g. relapsed or terminally ill patients) study tests may be omitted at the physician's discretion).

9.2 Research Related Procedures and Activities

Patients will be monitored for the persistence and function of UCB-derived regulatory T cells. Testing includes flow cytometry analysis of peripheral blood as shown in the table below. Immune cell subsets will include T regulatory and T effector cell populations.

Activity/ Laboratory	Upon Admission Pre Chemo	Days Post-UCBT			
		+0 Prior to cord blood infusion	+4, +7, +14 +28, (±3 days)	+60 +100 (±7 days)	180 (±30), 360 (±30), 720 (±30)
Evaluation of Treg cell infusion toxicity – per section 10.2		For 48 hours post-infusion			
Monitoring for early stopping rule events		Through Day +100 Treatment related mortality and Grade III/IV acute GVHD			
6 – 10 ml green top tubes 1 – 10 ml red top tube	X	X	X	X	X

Call TTL at 5-6165 for sample pick-up

It is recognized that with novel therapies as used in this study, the timing of protocol directed research samples may miss important patient specific events. For this reason, additional sets of research samples may be drawn at up to 3 time points that are not specified above.

Note: if a patient is not abiding by the required clinical care calendar (Section 9.1), the collection schedule of the toxicity data and research related samples may be altered or discontinued on an individual patient basis, as appropriate.

10 Adverse Event Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page http://evs.nci.nih.gov/ftp1/CTCAE/About.html

10.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious Adverse Event Or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- 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
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious 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

If either the IND sponsor or the investigator believes the event is life-threatening or serious, the event must be evaluated by the sponsor for expedited reporting (21CRF 312.32(a)).

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Major Clinical (Protocol/Patient) Minor Deviations as defined by the Masonic Cancer Center:

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB, FDA) as detailed in Section 10.3. For the IRB this is 5 business days from discovery. For studies under an IND, it is 7 or 15 calendar days.

10.2 Adverse Event Documentation

Due to the intentional clearing of the marrow with chemotherapy as preparation for the UCB transplantation, it is expected that all patients will experience severe depression of their blood counts and other related toxicities as detailed in appendix III – Expected Side Effects of the Preparative Regimen, UCB infusion and GVHD prophylaxis. Therefore, adverse event collection for the purposes of this study will focus on **targeted adverse events and unexpected adverse events within 48 hours of the Treg Cell Infusion**. If a subject discontinues treatment early, AEs will no longer be collected.

For the purposes of this study, targeted adverse events (Appendix VI) and unexpected events will be collected at the following time points in relation to the Treg Infusion:

- Prior to Treg infusion
- 1 to 4 hours after the Treg infusion
- 24 hours (±2 hours) after Treg infusion
- 48 hours (±2 hours) after Treg infusion
- 1 week after Treg infusion if ongoing grade 3 toxicity (grade 2 neurotoxicity) at the 48 hour assessment

In addition the following event outcomes impact the progress of the study and must be reported as an early **stopping rule event** per Section 10.3:

- Grade III or IV acute GVHD within 100 days of the cell infusion
- Treatment related mortality (TRM) by day 100 post-transplant

Refer to Section 12.4 for the full definition of the stopping rule events.

FOR UNLICENSED UCB UNITS ONLY: Selected expected adverse reactions determined to be caused by or at least possibly caused by the UCB units based on objective evidence will be reported in an expedited manner to the FDA under University of Minnesota IND BB-14797 (C. Brunstein, MD, PhD – sponsor/investigator).

10.3 Required Reporting: FDA, IRB and Masonic Cancer Center's SAE Coordinator

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers
U of MN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm For a complete list refer to http://www.research.umn.edu/irb/guidance/ae.html#.VC7xraI0-sh	Within 5 business days of event discovery	Report Form	irb@umn.edu
FDA	Unexpected and fatal or unexpected and life threatening suspected adverse reaction	no later than 7 Calendar Days#	MCC SAE	Submit to CBER as an amendment to IND
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	no later than 15 Calendar -Days#		
	All other events per CRF 312.33	At time of IND annual report	Summary format	Submit as part of the IND annual report
Masonic Cancer Center SAE Coordinator	Events that count toward the early study stopping rule.	At time of reporting	Event Form	SAE Coordinator mcc-saes@umn.edu

following the sponsor's initial receipt of the information

In each IND safety report, the sponsor must identify all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of the previous, similar reports.

The SAE Coordinator will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

11 Study Data Collection and Monitoring

11.1 Data Management

This study will collect regulatory and clinical data using University of Minnesota CTSI's instance of OnCore®(Online Enterprise Research Management Environment).

The Oncore database resides on dedicated secure and PHI compliant hardware consisting of 3 physical servers: dev, DR, and production. The dev server is located in the University of Minnesota (UMN) datacenter (WBOB) and houses six database instances (test, train, sandbox, mcc reports, oncdm, and vendor) that are backed up locally because the data is refreshed from Oncore production data. The production server is located in the UMN datacenter (WBOB). All the data servers are managed by the Academic Health Center – Information Systems (AHC-IS) virtual servers which utilize clustered infrastructure to provide real-time failover of virtual servers. This real-time clustering is physically limited to the UMN data center. All relevant AHC IS procedures related for PHI compliant servers (as required by the Center of Excellence for HIPAA Data) apply to Oncore databases.

The integrated data will be stored in PHI compliant servers managed by AHC IS with access given to those authorized users in the Clinical and Translation Science Institute Informatics team (CTSI BPIC and MCC CISS). The data will be integrated and extracted to researchers through the CTSI Informatics team and will be delivered through secure and compliant mechanisms (e.g. AHC IE data shelter, BOX, sftp, etc). If data de-identification is needed, then compliant AHC IE data de-identification tools will be used. The informatics team will grant the IRB approved study team members access to data.

Additional immune monitoring data about correlative laboratory samples generated by the Masonic Cancer Center Translational Therapy Laboratory (TTL) from the protocol-directed correlative research samples is stored in their Laboratory Information Management System (LIMS). The LIMS database application is also stored on a production server located in the UMN datacenter (WBOB) and is managed by the Academic Health Center.

Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore.

11.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRF) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Primary Clinical Research Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRF based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

11.3 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp.

For the purposes of data and safety monitoring, this study is classified as high risk (under a locally held IND). Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the study's progress at least quarterly with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in Section 10.3 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.

11.4 IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the sponsor-investigator with assistance from the MCC Clinical Trials Office (CTO) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

11.5 Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the sponsor-investigator and/or sponsor/investigator designee, IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of

applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

11.6 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at 6 years after the study file is closed with the IRB and FDA.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO before destroying any study related records.

12 Statistical Considerations

12.1 Study Design and Endpoints

This is a single center pilot study that will help with the design of a future full scale study of the efficacy and safety of our method by determining feasibility and providing estimates and variances of the endpoints for both safety and efficacy.

Feasibility will be determined by demonstration of a workable protocol, including the proportion of patients advancing from enrollment to receipt of the full course IL-2. (This endpoint was no longer applicable as of the January 2018 protocol version which removed IL-2 from the treatment plan due to unacceptable toxicity)

Safety

- Probability of grade II-IV aGVHD
- Probability of grade III-IV aGVHD
- Probability of treatment related mortality (TRM) at 6 months
- Probability of relapse at 1 year
- Probability of viral and fungal infections by 1 year
- Pattern of immune reconstitution

The proportion of patients with detectable Treg cells at Day 14 post infusion

Efficacy

- Length of Treg survival after infusion of Treg
- Probability of grade II-IV aGVHD

- Probability of relapse at 1 year
- Probability of viral and fungal infections by 1 year
- Pattern of immune reconstitution
- The proportion of patients with detectable Treg cells at Day 14 post infusion

Transplant Related Objectives

- Determine the incidence of chimerism at Day 100
- Determine the probability of survival at 1 year
- Determine the incidence of neutrophil recovery at Day 42
- Determine the incidence of platelet recovery at 1 year
- Determine the incidence of chronic GVHD at 1 year

12.2 Statistical Analysis

Analysis will primarily be descriptive. Safety, efficacy and transplant-related endpoints will be evaluated with descriptive statistics and plots or cumulative incidence curves if enough evaluable patients are available for time-to-event endpoints. If censoring or competing risk is an issue in cumulative incidence of GVHD, relapse, infection and engraftment, non-event death will be treated as a competing risk. Likewise, relapse will be treated as a competing risk in estimation of TRM. If sufficient patients are evaluable, survival will be estimated by Kaplan-Meier curves. The primary efficacy variable is the length of Treg survival after Treg infusion. Continuous endpoints such as this will be described by medians, ranges and interquartile ranges as well as means and standard deviations if normally distributed. Categorical endpoints will be described with simple proportions and descriptive plots.

12.3 Sample Size Justification

Due to the nature of the pilot study and allowable resources, study accrual will stop when a total of 10 treated patients have been enrolled.

12.4 Monitoring Guidelines (Stopping Rule Events)

Early stopping rules for treatment related morality (TRM) and Grade III-IV Acute Graft versus Host Disease (AGVHD) by Day 100 will be in place to continuously monitor safety. In the event that a monitoring boundary is triggered, study enrollment will be suspended and the PI and IRB will be notified.

The stopping rules for TRM and AGVHD were developed using an adaptation of Pocock stopping boundaries (28).

Treatment Related Mortality

Treatment related mortality (TRM) is defined as death due to any cause other than relapse by day 100 after transplant. The study will stop and the protocol will be re-evaluated if the rate exceeds the boundary. We expect that the rate of TRM will be about 25%. The goal is to construct a boundary based on mortality such that the probability of early stopping is at most 10% if the rate is equal to 25% and our sample size is 10. With these stipulations, the trial will be stopped and reviewed if 3/4, 4/6, 5/9 or 6 patients have TRM. The probability of hitting the stopping boundary if the rate is 60% is 77%.

Grade III-IV AGVHD

Grade III-IV AGVHD will be monitored through day 100 after transplant. The study will stop and the protocol will be re-evaluated if the rate exceeds the boundary. We expect that the rate of AGVHD will be about 20%. The goal is to construct a boundary based on AGVHD such that the probability of early stopping is at most 10% if the rate is equal to 20% and our sample size is 10. With these stipulations, the trial will be stopped and reviewed if 2/2, 3/4, 4/8 or 5 patients have AGVHD. The probability of hitting the stopping boundary if the rate is 50% is 72%.

13 Conduct of the Study

13.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

13.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

13.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

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Appendix I – Eligibility Checklist

Adoptive Transfer of T Regulatory Cell for Suppression of Acute Graft-vs-Host-Disease after an Umbilical Cord Blood Transplant for Hematologic Malignancies

Eligibility Checklist – page 1 of 2

Patient initials ☐☐☐☐ (1st 2 initials of first name + 1st 2 initials of last name) Patient ID☐☐ (Seq # (i.e. 01, 02, 03, etc.))

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the patient from study entry.

		Yes	No
1.	Must be ≥18, but < 70 years of age with no matched 7/8 or 8/8 sibling donor - patients ≥ 70 and ≤ 75 years of age may be eligible if they have a Co-Morbidity score ≤ 2 (http://www.qxmd.com/calculate-online/hematology/hct-ci)	☐	☐
2.	UCB unit(s) composing the graft will be selected according to the current University of Minnesota umbilical cord blood graft selection algorithm and an additional cord blood unit to be used as the source to manufacture the Treg product. This UCB unit must be matched at 4-6/6 to the patient, considering HLA-A, B at the antigen level and DRB1 at the allele level	☐	☐
3.	**Acute Leukemias:** Must be in remission by morphology (around 5% blasts, but < 20% blasts by morphology). Also a small percentage of blasts that is equivocal between marrow regeneration versus early relapse are acceptable provided there are no associated cytogenetic markers consistent with relapse. Refer to Section 5.2 for complete definitions. **Burkitt's Lymphoma** in CR2 or subsequent CR **Natural Killer Cell Malignancies** **Chronic Myelogenous Leukemia:** all types except refractory blast crisis. Chronic phase patients must have failed at least two different tyrosine-kinase inhibitors (TKIs), or been intolerant to all available TKIs or have *T315I* mutation. **Myelodysplastic Syndrome:** IPSS INT-2 or High Risk; R-IPSS High or Very High; WHO classification: RAEB-1, RAEB-2; Severe Cytopenias: ANC < 0.8, Anemia or thrombocytopenia requiring transfusion; Poor or very poor risk cytogenetics based on IPSS or R-IPSS definitions; therapy-related MDS. Blasts must be < 5% by bone marrow aspirate morphology. If ≥5% blasts, patient requires chemotherapy for cytoreduction to <5% blasts prior to transplantation. **Large-Cell Lymphoma, Hodgkin Lymphoma and Multiple Myeloma** with chemotherapy sensitive disease that has failed or patients who are ineligible for an autologous transplant. **Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL), Marginal Zone B-Cell Lymphoma, Follicular Lymphoma**, which have progressed within 12 months of achieving a partial or complete remission. Patients who had remissions lasting > 12 months are eligible after at least two prior therapies. Patients with bulky disease should be considered for debulking chemotherapy before transplant. Patients with refractory disease are eligible, unless has bulky disease and an estimated tumor doubling time of less than one month. **Lymphoplasmacytic Lymphoma, Mantle-Cell Lymphoma, Prolymphocytic Leukemia** are eligible after initial therapy if chemotherapy sensitive. Patients must have undergone an autologous transplant ≤ 12 months prior to transplant on this study or have received multi-agent or immunosuppressive chemotherapy within 3 months of the preparative regimen.	☐	☐

Adoptive Transfer of T Regulatory Cell for Suppression of Acute Graft-vs-Host-Disease after an Umbilical Cord Blood Transplant for Hematologic Malignancies

Eligibility Checklist – page 2 of 2

Patient initials ☐☐☐☐

INCLUSION CRITERIA (continue)

A "NO" response to any of the following disqualifies the patient from study entry.

		Yes	No
4.	Karnofsky score ≥ 70%	☐	☐
5.	Adequate organ function within 14 days (30 days for cardiac and pulmonary) of registration on-study defined as: ▪ Renal: creatinine ≤ 2.0 mg/dL, for patient with a creatinine > 1.2 mg/dL or a history of renal dysfunction an estimated glomerular filtration rate ≥ 40 mL/min/1.73 m^2 is required ▪ Hepatic: AST, ALT, alkaline phosphatase ≤ 5 x upper limit of normal and total bilirubin ≤ 2.5 mg/dL except for patients with Gilbert's syndrome or hemolysis ▪ Pulmonary function: DLCO, FEV_1, FVC ≥ 40% predicted, and absence of O_2 requirements. • Cardiac: Absence of decompensated congestive heart failure, or uncontrolled arrhythmia and left ventricular ejection fraction ≥ 40%.	☐	☐
6.	Sexually active females of childbearing potential and males with partners of child-bearing potential must agree to use adequate birth control during study treatment	☐	☐
7.	Voluntary written consent	☐	☐

EXCLUSION CRITERIA

A "YES" response to any of the following disqualifies the patient from study entry.

		Yes	No
8.	Untreated active infection	☐	☐
9.	History of HIV infection	☐	☐
10.	Pregnant or breast feeding	☐	☐
11.	Prior allogeneic transplantation	☐	☐
12.	Less than 3 months from myeloablative conditioning for autologous transplantation	☐	☐
13.	Evidence of progressive disease by imaging modalities or biopsy - persistent PET activity, though possibly related to lymphoma, is not an exclusion criterion in the absence of CT changes indicating progression.	☐	☐
14.	CML in blast crisis	☐	☐
15.	Large cell lymphoma, mantle cell lymphoma and Hodgkin disease that is progressing on salvage therapy.	☐	☐
16.	Active central nervous system malignancy	☐	☐

Having obtained consent and reviewed each of the inclusion/exclusion criteria, I verify that this patient is eligible

_____ _____

Signature of enrolling physician Date

Appendix II – Karnofsky Performance Status

Karnofsky Performance Scale	
Percent	**Description**
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

Appendix III – Expected Risks of the Preparative Regimen, GVHD Prophylaxis and UCB Transplant

Preparative Regimen:

Cyclophosphamide		
common	**less common**	**rare, but may be serious**
• low white blood cell count with increased risk of infection • hair loss or thinning, including face and body hair (usually grows back after treatment) • nausea • vomiting • loss of appetite • sores in mouth or on lips • bleeding from bladder, with blood in urine • diarrhea • long-term or short-term infertility (inability to have children) in women and men	• low platelet count (mild) with increased risk of bleeding • darkening of nail beds • acne • tiredness • infection • fetal changes if you become pregnant while taking cyclophosphamide	• heart problems with high doses, with chest pain, shortness of breath, or swollen feet • severe allergic reactions • skin rash • scarring of bladder • kidney damage (renal tubular necrosis) which can lead to kidney failure • heart damage, with trouble getting your breath, swelling of feet, rapid weight gain • scarring of lung tissue, with cough and shortness of breath • second cancer, which can happen years after taking this drug • death from infection, bleeding, heart failure, allergic reaction, or other causes

Fludarabine		
common	**less common**	**rare, but may be serious**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with tiredness and weakness • tiredness (fatigue) • nausea • vomiting • fever and chills • infection	• pneumonia • diarrhea • loss of appetite • weakness • pain	• numbness and tingling in hands and/or feet related to irritation of nerves • changes in vision • agitation • confusion • clumsiness • seizures • coma • cough • trouble breathing • intestinal bleeding • weakness • death due to effects on the brain, infection, bleeding,

Fludarabine		
common	**less common**	**rare, but may be serious**
		• severe anemia, skin blistering, or other causes • death from infection, bleeding, heart failure, allergic reaction, or other causes

Total Body Irradiation		
common	**less common**	**rare, but may be serious**
• nausea and vomiting • diarrhea • cataracts • sterility • endocrinopathies • growth failure • intestinal cramps • mucositis	• parotitis • interstitial pneumonitis • generalized mild erythema • veno-occlusive disease	• dysphagia • vertebral deformities • nephropathy • risk of 2nd malignancy years later (when given along with chemotherapy)

GVHD Prophylaxis:

Mycophenolate Mofetil (MMF)	**Sirolimus (Rapamycin)**
• pancytopenia • headache • insomnia • electrolyte imbalances • leg cramps/bone pain • hypertension • dizziness • hyperglycemia • rash • nausea • diarrhea	• fast heart rate • pain when breathing, feeling short of breath • chest pain, feeling weak or tired • coughing up blood or mucus • feeling like you might pass out • pale skin, easy bruising or bleeding, weakness • fever, chills, body aches, flu symptoms • night sweats, weight loss • swelling of face, stomach, hands or feet • rapid weight gain • pain or burning when urinating • slow healing of a wound • joint pain • nausea, vomiting, diarrhea, constipation, stomach pain • headache • acne or skin rash • high triglycerides and cholesterol

Umbilical Cord Blood Transplant:

- nausea and vomiting
- allergic infusion reaction (including itching, hives, flushing [red face], shortness of breath, wheezing, chest tightness, skin rash, fever, chills, stiff muscles, or trouble breathing)
- graft-versus-host-disease (GVHD)
- veno-occlusive disease,
- mucositis,
- infections (sepsis)
- failure to engraft

To improve engraftment G-CSF will be administered beginning Day +5

G-CSF	
Less Common	**Rare, but may be serious**
• bone or muscle pain • injection site reaction (redness, pain, or swelling)	• allergic reaction • spleen enlargement or rupture –symptoms of an enlarged spleen include a feeling discomfort, fullness, or pain on the upper left side of the abdomen; this pain may spread to the left shoulder • serious lung problems (ARDS) • coughing up blood

Appendix IV – GVHD Scoring

Acute GVHD

Organ involvement will be staged using the criteria outlined in the table below. Biopsy of each organ site at diagnosis or major change in disease activity will be performed unless clinical circumstances make it impossible.

Consensus Clinical Stage and Grade of Acute GVHD (Przepiorka *et al*, 1995)

Stage	Skin	Liver	Lower Gastrointestinal Tract	Upper Gastrointestinal Tract
1	Maculopapular rash <25% of body surface	Bilirubin 2.0 – 3.0 mg/dl	Diarrhea 500 – 1000 mL/day or 280 – 555 mL/m^2	No protracted nausea and vomiting
2	Maculopapular rash 25-50% body surface	Bilirubin 3.1 – 6.0 mg/dl	Diarrhea 1000 – 1500 mL/day or 556 – 833 mL/m^2	Persistent nausea, vomiting or anorexia
3	Generalized erythroderma	Bilirubin 6.1 – 15.0 mg/dl	Diarrhea >1500 mL/day or >833 mL/m^2	
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dl	Severe abdominal pain, with or without ileus, or stool with frank blood or melena	

Grading for Treatment Criteria:

Mild GVHD Skin stage I-II only (Equivalent to Seattle Grade I).

Moderate GVHD Skin stage I-III and/or liver I-IV and/or Gastrointestinal tract (GI) I-III and/or Upper GI (UGI). (Equivalent to Seattle Grade II, III).

Severe GVHD Any stage IV along with severe clinical illness.

Late Acute and Chronic GVHD

Late acute and chronic GVHD will be assessed using the National Institutes of Health (NIH) Consensus Criteria.

Appendix V – Targeted Toxicity

Patient Initials: _____ Date of Assessment: _____ Assessment Time point: _____

ADL = activities of daily living CTCAE v 4.0 Refer to Section 10.2 for time points.

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Infusion related reaction	None	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment; prophylactic medications indicated for ≤ 24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion);	Life-threatening consequences; urgent intervention indicated
Dyspnea	None or no change	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest;limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Hypoxia	None	……	Decreased O_2 saturation with exercise (e.g., pulse oximeter < 88%) intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter < 88% or PaO_2 ≤ 55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)
Fever	None	38.0 - 39.0 degrees C (100.4 -102.2 F)	> 39.0 - 40.0 degrees C (102.3 - 104.0 degrees F)	> 40.0 degrees C (>104.0 degrees F) for ≤ 24 hours	> 40.0 degrees C (>104.0 degrees F) for > 24 hours
Febrile neutropenia		……	……	temperature of >38.3 degrees C (101 degrees F) or a temp of ≥38 degrees C (100.4 degrees F) for > 1 hour	Life-threatening consequences; urgent intervention indicated
Chills	None	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	……
Hypertension	None	Pre-hypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg)	Stage 1 hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 - 99 mm Hg); medical intervention indicated; recurrent or persistent ≥ 24 hrs); symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously WNL; monotherapy indicated	Stage 2 hypertension (systolic BP ≥ 160 mm Hg or diastolic BP ≥ 100 mm Hg); medical intervention indicated; > drug or more intensive therapy than previously used indicated.	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated.
Hypotension	None	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated
Edema	None	Localized to dependent areas, no disability or functional impairment	Moderate localized edema and intervention indicated; limiting instrumental ADL	Severe localized edema and intervention indicated; limiting self care ADL	……
Pneumonitis	None	Asymptomatic; clinical or diagnostic observations only;	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g. intubation or tracheotomy)
Fatigue	None	Fatigue relieved by rest	Fatigue not relieved by rest; limiting ADL instrumental	Fatigue not relieved by rest; limiting self care	……
Rash	None	Covering < 10% body surface area (BSA)	Covering 10-30% body surface area (BSA)	>30% body surface area (BSA)	Generalized exfoliative, ulcerative, or bullous dermatitis
Weight Gain	None	5 - <10% from baseline	10 - <20% from baseline	≥20% from baseline	……

Person Completing Form: _____