Coversheet

Protocol and Statistical Analysis Plan

Official Study Title	**T-CELL RECEPTOR (TCR) ALPHA BETA+/CD19+ DEPLETION IN HAPLOIDENTICAL ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (ALLO-HCT) FOR ADULT AND PEDIATRIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND NON-MALIGNANT DISORDERS (HAPLOTAB)**
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T-Cell Receptor (TCR) αβ+/CD19+ Depletion in Haploidentical Allogeneic Hematopoietic Cell Transplantation (allo-HCT) for Adult and Pediatric Patients with Hematological Malignancies and Non-malignant Disorders

(HAPLOTAB)

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HAPLOTAB CHECKLIST FOR PATIENT ELIGIBILITY FOR TREATMENT

Patient ID: _____ Patient Name: _____

The following must be answered "**YES**" for a patient to be eligible to participate on this study:

YES	NO	VALUE/DATE	
__	__		Age less than or equal to 55 years
__	__		Lack of suitable conventional donor (as defined in protocol) or presence of rapidly progressive disease not permitting time to identify an HLA-matched unrelated donor
__	__	___/___	Lansky/Karnofsky score > 50
__	__	___/___	Signed written informed consent
__	__		Diagnosis of one of the following: (check one)

- ☐ Patient with life threatening hematological malignancy including "high-risk" ALL in first complete remission (CR1); ALL in second or subsequent remission (≥ CR2); high-risk AML in CR1; AML in second or subsequent CR; myelodysplastic syndromes (MDS); non-Hodgkin's lymphomas (NHL) in second or subsequent remission (≥ CR2); CML
- ☐ Hemophagocytic Lymphohistiocytosis (HLH) including familial HLH, relapsed HLH or CNS HLH
- ☐ Primary Immunodeficiency Disorders (PID)
- ☐ Hemoglobinopathies including thalassemia or sickle cell disease (SCD)
- ☐ Severe aplastic anemia (SAA) not responding to immune suppressive therapy
- ☐ Congenital/hereditary cytopenias including Fanconi anemia (FA) without malignant clonal evolution (MDS, AML)
- ☐ Other inherited bone marrow failure syndromes (IBMFS)
- ☐ Severe chronic active Epstein Barr virus infection (SCAEBV) with predilection for T- or NK-cell malignancy

The following must be answered "**NO**" for patient to be eligible

YES	NO	VALUE/DATE	
__	__	___/___	Life expectancy of ≤ 6 weeks limited by disease other than leukemia.
__	__	___/___	Greater than grade II acute graft versus host disease (GVHD) or chronic extensive GVHD due to a previous allograft at the time of inclusion
__	__	___/___	Subject receiving an immunosuppressive treatment for GVHD treatment due to a previous allograft at the time of inclusion
__	__	___/___	Symptomatic cardiac disease OR left ventricular shortening fraction < 25% or ejection fraction < 40%
__	__	___/___	Severe renal disease, with creatinine clearance < 40cc/1.73 m2
__	__	___/___	Pre-existing severe restrictive pulmonary disease, FVC < 40% of predicted
__	__	___/___	Severe hepatic disease with ALT/AST ≥ x2.5 upper limit of normal or bilirubin level ≥ x1.5 upper limit of normal
__	__		Serious concurrent uncontrolled medical disorder or mental illness
__	__		Pregnant or breastfeeding female subject
__	__		Current active infectious disease including viral and fungal diseases at the time of enrollment; that on evaluation of PI precludes ablative chemotherapy or successful transplantation.
__	__		Active HIV infection
__	__		Severe personality disorder or mental illness that would preclude compliance with the study

To register patients and check eligibility, call Dr. Morales at 832-826-0860.

_____ _____

Signature of Enrolling Investigator Date

1.0 OBJECTIVES

1.1. This is a phase I/II study evaluating the safety and feasibility of αβ T cell-depleted (TCD) haploidentical (or partially mismatched) hematopoietic cell transplantation (HHCT) by negative selection of T cell receptor alpha/beta (TCR αβ+) T cells and CD19+ depletion using an automated CliniMACS device in pediatric and adult patients

Primary Endpoints:

1. Cumulative incidence of both neutrophil and platelet engraftment by day 42 post HCT as a composite endpoint.
2. Cumulative incidence of Grade III or higher acute graft versus-host disease (aGVHD) by day 100 post HCT

Secondary Endpoints:

1. Cumulative incidence of transplant-related mortality (TRM) at Day 100 and Day 365
2. Overall survival (OS) up to one year post HCT
3. Cumulative incidence of chronic graft versus-host disease (GVHD) up to 2-years post-HCT

Exploratory Objectives:

1. Feasibility of using the automated CliniMACS system to produce a graft with defined cell composition
2. Incidence of viral reactivations – defined as detectable viral load by peripheral blood polymerase chain reaction (PCR) – and/or symptomatic infections up to one year post HCT
3. For patients with hematological malignancies, complete donor chimerism within 30 days from engraftment.
4. T-, B- and NK-cell subset analysis at Day 30, 60, 100, 180 and 365 post-HCT
5. Recent Thymic Emigrants (RTE) at Day 100, provided absolute lymphocytes count (ALC) is > 200 x10e9/L, then at Day 180 and 365
6. T-cell repertoire analysis by flow cytometry at Day 180 post-transplant
7. Proportion of alpha/beta versus gamma/delta T-cell receptor positive cells present at Day 30, 60, 100, 180 and 365 post-HCT
8. NK screening subset at Day 30, 60, 100, 180 and 365 post-HCT
9. Extended NK cell phenotype at Day 30, 60, 100, 180 and 365 post-HCT

2.0 BACKGROUND AND RATIONALE

2.1. Introduction

While most hematopoietic cell transplant (HCT) candidates have an acceptable donor, regarded as an HLA-matched or minimally mismatched donor, some patients lack an optimal donor, that is, donor who is matched at high resolution at HLA-A, HLA-B, HLA-C and HLA-DRB1. The likelihood of finding an optimal donor varies among racial and ethnic groups with the highest probability among whites of European descent, at 75%, and the lowest among Blacks of South or Central American descent, at 16%. Likelihoods for other groups are intermediate. Few

patients will have an optimal cord-blood unit (CBU) – that is, one matched at the antigen level at HLA-A and HLA-B and at high resolution at HLA-DRB1. [1]

Allogeneic haploidentical hematopoietic cell transplantation (allo-HHCT) using contemporary approaches provides similar outcomes to transplantation from matched unrelated donor (MUD) or CBU. [2-4]

HHCT platforms based on T cell depletion rely mainly on graft processing to achieve an ideal graft composition that prevents rejection, recurrence of leukemia, infection and GVHD without requiring post-transplant immune suppressive therapy. T cell depletion-based graft require dedicated laboratories and are more expensive than conventional unmanipulated HCT, especially if combined with adoptive transfer of T cell populations chosen to improve post-transplant immune reconstitution. However unlike unmanipulated HHCT, pharmacologic GVHD prophylaxis and further treatments are not necessary in T cell depleted haploidentical transplants thus reducing the need for and the cost of supportive care and post-transplant hospitalizations.

2.2. Evolution of the Methodology of T cell Depletion Haploidentical HCT

Various strategies have been attempted to promote successful utilization of allo-HHCT. Earlier strategies involved CD34+ selection in the transplanted allo-graft.[5,6] This allowed for the indirect removal of alloreactive T cells responsible for graft-versus-host disease (GVHD), and for the indirect removal of B cells which can cause post-transplant lymphoproliferative disorder (PTLD) and are also thought to play a role in the pathogenesis of chronic GVHD (cGVHD), thus removal of B cells may reduce the risk of GVHD.[7-9] In a landmark phase II study of haploidentical transplant using CD34+ immunoselection for patients with high risk acute leukemia, the group demonstrated high engraftment rates (93%) and high rate of GVHD prevention.[5] Even with low rates of GVHD, the leukemia relapse in this, and other large studies, was not increased in high-risk leukemia patients. Despite lack of a potent T cell mediated graft versus leukemia (GVL) effect with T cell depletion (TCD), eradication of residual leukemia cells was still achieved. This may have been mediated by, a) the myeloablative nature of the conditioning regimen, b) subclinical GVL effect by the residual T cells within the graft being unopposed by any post-transplant immunosuppressive therapy, as well and c) T cell independent GVL effect of donor NK cells preserved within the graft.

Patients who received TCD HHCT – or similarly with *in vivo* depletion using serotherapy – exhibit a very narrow T cell repertoire leading to prolonged susceptibility to serious opportunistic infections. In the study by Aversa and colleagues, 27 of 103 patients died from serious infections. Infection-related mortality has been the main cause of transplant failure using this approach. [10-12] In order to improve immunological reconstitution and minimize risk of severe, life-threatening infections, successful strategies for adoptive T cell therapy were developed; most notably, adoptive transfer of pathogen specific T lymphocytes.[13] Across different studies, donor-derived virus-specific T cells (VSTs) were shown to have a response rates in 70-90% of recipients; similarly, in closely matched third party VSTs, response rates were between 60-70%.[14] Other strategies of delayed T cell addback using adoptive T cell therapy included allodepletion and alloanergy

induction, immune modulation by co-infusion of regulatory cell populations and the use of safety switch gene-modified T cells. [15-20]

To improve clinical outcomes, the CD34-positive selection approach was largely replaced by negative selection of peripheral blood progenitor cells (PBPCs). This approach allows for the preservation of other immune cells – such as Nk cells, dentritic cells and monocytes – supporting engraftment and immune recovery. One successful application of this strategy involves selective depletion of CD3+ T cells (+/- CD19+ B-cells) from the grafts via immunomagnetic beads using the automated CliniMACS device (Miltenyi Biotec, Germany).

This method of transplantation was described by Hale et al. in a study of 20 pediatric patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), or non-Hodgkin lymphoma (NHL) using a conditioning regimen of total body irradiation (TBI), cyclophosphamide, thiotepa and rabbit antithymocyte globulin (ATG).[21] Three subsequent trials were completed in patients with various hematological malignancies using a reduced intensity conditioning (RIC) regimen consisting of fludarabine, melphalan, thiotepa and OKT3.[22-24] Rates of engraftment were favorable in these studies ranging from 83% to 97%. In the study by Hale et al., the rate of acute GVHD (aGVHD) was 16% (all grade I-II) with the rate of cGVHD (all limited cases) being 5.2%. [21] The trials using RIC regimens had higher rates of aGVHD, though mostly lower grade (grade I-II ranged from 24% to 39%, with grade III-IV in only 3%-14%). In these studies, the rate of cGVHD ranged from 10%-28%. The rate of transplant-related mortality (TRM) in one study using RIC regimen was only 2.6%.[23] However TRM remained significant in the other studies ranging from 20-30%.[22]

In more recent years, Bader et al. reported on use of grafts depleted of T and B cells using CD3- and CD19-coated microbeads and automated CliniMACS device. Children with acute leukemia received conditioning consistent of fludarabine, thiotepa, melphalan and OKT3 or ATG. Rate of primary engraftment was 88%, aGVHD grade II occurred in 20% and grade III-IV in 7%; cGVHD occurred in 21%. Non-relapse mortality (NRM) was 8% at 1-year and 20% at 5-years.[25] Using the same approach, but with RIC regimens in 61 adults, the incidence of grade II-IV acute and chronic GVHD was 46% and 18% respectively. Day 100 mortality was 23% and at 2 years 42%. Relapse rate was 31% and overall survival (OS) at 2 years was 28%.[26] A major concern with this approach was the high incidence of GVHD.

To overcome this problem, Chaleff et al. described a large-scale clinical method using the Miltenyi Biotec CliniMACS® T-cell receptor alpha/beta (TCR α/β) system for the depletion of α/β T lymphocytes from peripheral blood stem cells while retaining all other cells. [27] The CliniMACS® TCR α/β system uses murine monoclonal antibodies specific for the T cell receptor α/β antigen conjugated to biotin in combination with the CliniMACS® anti-Biotin reagent. The pioneering experience of the Handgretinger's group showed that TCR αβ/CD19 depletion allows a T cell reduction of 4.5-5 log which is comparable to CD34+ selection methods.[28,29] It also ensures patients to receive NK cells, monocytes and dendritic cells, and most important, the TCR gamma/delta+ (γδ+) T lymphocytes. TCR γδ T cells appear to exert anti-leukemic activity since they directly recognize stress-

induced self-antigens expressed by malignant cells. Strikingly, they do not recognize specific processed peptide antigens as presented on major histocompatibility (MHC) molecules and so are not expected to induce GVHD.[30,31]

Early clinical experiences with children transplanted using this approach showed excellent full-donor engraftment, a rapid early expansion of donor-derived TCR γδ+ T cells that contributed to a very fast immunological reconstitution.[28] Using the same method of graft engineering, Locatelli and colleagues achieved similar results in terms of engraftment, prevention of both acute and chronic GVHD, and a rapid recovery of post-transplant immunity in children independently from the conditioning regimen, whether TBI based (children with leukemia) or chemotherapy based (children with non-malignant disorders).[32,33] In 23 children with non-malignant disorders, no cases of visceral acute or chronic GVHD were observed and survival was 91% at 2 years. [34] The same group in Italy and others have tested in phase 1/2 clinical trials, the use of a chimeric gene incorporating the death domain of inducible caspase 9 (iC9) in children with either malignant or non-malignant disorders who receive TCR α/β/CD19 depleted haplo-HCT followed by infusion of titrated numbers of iC9 T cells on day 14 +/- 4. The goal was to further improve immune reconstitution without increasing the risk of GVHD. These modified T cells can be rapidly eliminated by the administration of AP1903 if acute GVHD occurs.[20,35] TCR γδ+ T cells recovering during the first year after HCT in 102 patients with acute leukemia correlated with a reduced incidence of infection in the study by Perko et al.[36] Children with an elevated number of TCR γδ+ T cells post engraftment experienced only viral infections, while low/normal TCR γδ+ T cell group had viral, bacterial and fungal infections. Enhanced TCR γδ+ T cell recovery resulted in higher event-free survival (EFS) rate in 1 year.

Outcomes of TCR α/β/CD19 depleted haplo-HCT were evaluated in a cohort of children with chemo-refractory AML. The conditioning regimen included fludarabine and cytarabine (cytoreduction phase) followed by treosulfan and thiotepa (myeloablative phase). Tocilizumab was given instead of ATG in all patients, abatacept in 10 patients. Post-transplant CD45RA-depleted donor lymphocytes were given prophylactically; with or without a hypomethylating agent. Overall results were promising with 95% of patients achieving a complete remission, 18% having a grade II-IV acute GVHD and 23% chronic GVHD. At 2 years, NRM was 9%, relapse rate 42%, EFS and OS were 49% and 53% respectively.[37] More recently, the advantages of this strategies were demonstrated in 20 patients with sickle cell disease (SCD) (children and adults, median age 15 years). Conditioning consisted of ATG, thiotepa fludarabine, and treosulfan. Two patients succumbed to a cytomegalovirus (CMV) pneumonitis and a macrophage activation syndrome. One patient required renal replacement therapy for BK virus nephritis. None developed grade III-IV GVHD. At a median follow up of 21 months (range 9-62 months), overall survival was 90% documenting the feasibility, safety and efficacy of TCD haplo-HCT also for advanced stage SCD patients.[38]

Prezioso et al reported on transplant outcome using this approach in 59 adults (median age 48 years, range 19–74) with hematological malignancies.[39] All patients were conditioned with ATG (median at Day -10), treosulfan, fludarabine, and thiotepa. No additional pharmacologic prophylaxis for GvHD was given after transplantation. Full donor engraftment was achieved in 56/59 (95%) patients. Two

patients developed severe GVHD resulting in death. One of them had received the highest dose of ab+ T cells (3.7×10^5/kg). Skin limited grade II acute GvHD was observed in 8 patients who responded rapidly to steroids. At the time of this report, only two patients have developed chronic GvHD that responded to steroid and cyclosporine treatment. Interestingly, naïve and memory T-cell subsets increased significantly over the first year after transplantation. Similarly, serum immunoglobulin levels normalized within 3 months. In two patients, CMV reactivation was associated with a significant expansion of pathogen specific CD8+ T cells that contributed to spontaneous elimination of viral load. Disease relapse was the main cause of death in 16/59 patients; 15 patients died without relapsing, 11 of them from infections.

Similar results reported by a team in Turkey in 34 adult patients with either AML(n=24) or ALL (n=10). [40] Conditioning regimen consisted of thiotepa, melphalan, fludarabine, and ATG. Full donor chimerism was achieved in 31/34 patients. Four patients developed severe GvHD (2 acute, 2 chronic). A low NRM (11.7%) at day 100 was attributed to a rapid T-cell reconstitution. Relapse was the main cause of death (56.3%). DFS at 1 year was 42%.

2.3. Role of Natural Killer (NK) cells

Earlier investigations in the allo-HCT setting demonstrated several favorable actions of NK cells in grafts including facilitating engraftment, graft versus tumor (GVT) effects, and activity against opportunistic infections. Tumor cell lysis by NK cells can be mediated by mismatch of donor NK cell killer immunoglobulin-like receptors (KIRs) and KIR ligands on recipient cells, which facilitates GVL effect.[41-43] Transplantation from NK-alloreactive donors (n= 51) was associated with a significantly lower relapse rate in the 61 patients (3 vs. 47%) ($P > 0.003$) and better event-free survival (EFS) (67 vs. 18%, $P = 0.02$) in a cohort of 112 patients with high-risk AML.[10] Furthermore, the group from Madrid, Spain reported on $CD56^{dim}/CD56^{bright}$ NK cell ratio early post HHCT using TCR α/β/CD19 depleted grafts and outcome. In this prospective analysis of 60 patients undergoing 63 transplants for ALL, AML or other hematological malignancies (23 patients in CR1, 20 in CR2 and 20 in > CR2), the probability of relapse was 32 +/- 6% and disease free survival (DFS) was 52 +/- 6% with a median follow-up of 28 months. A high $CD56^{dim}/CD56^{bright}$ NK cell ratio was associated with better DFS (≥3.5; 77 ± 8% vs. <3.5; 28 ± 5%; p = 0.001) due to lower relapse incidence (≥3.5; 15 ± 7% vs. <3.5; 37 ± 9%; p = 0.04). Thus, a rapid expansion of “mature” NK cells post HHCT, and lower risk of relapse, further supports an NK cell-mediated GVL effect. [44]

2.4. Current Outcome Data for TCR αβ+ CD19+ Depletion

2.4.1. Graft Composition

Overall, studies have shown good results with respect to graft composition, achieving adequate depletion of TCR αβ+ CD19+ cells and good CD34+ cell recovery. Several pediatric studies have shown median numbers of CD34+ cells per kg body weight in the graft ranging from 7.9 $x10^6$ to 18.7 10^6. [45-51] Studies conducted have shown comparable median numbers of CD34+ cells per kg body weight of 11 $x10^6$ to 17.69 $x10^6$. Earlier studies

involving CD34+ selection for haploidentical transplant were notable for CD34+ cell counts/kg body weight ranging from 1.2 $x10^6$ to 13.8 $x10^6$. [5,6] Thus, the cell dose achieved with TCR αβ+ CD19+ depletion is not inferior to CD34+ selection. Overall, median numbers of αβ+ T cells and B cells per kg body weight ranged from 11 $x10^3$ to 55 $x10^3$ and 40 $x10^3$ to 300 $x10^3$, respectively, reflecting optimum depletion, typically on the order of 3-4 log depletion.[34,45,47,48,50] Median numbers of γδ+ T cells and NK cells in the graft range from 4 $x10^6$ to 11 $x10^6$ per kg body weight [34,40,45,47,52] and 22 $x10^6$ to 81.3 $x10^6$,[34,47-50] respectively.

2.4.2. **Engraftment**

Several studies have shown successful primary engraftment rates (83-100%) of TCR αβ/CD19+ depleted stem cell grafts for HHCT, with average absolute neutrophil count (ANC) recovery by day 10-15 and average platelet recovery by day 10-17. Although various conditioning regimens have been studied (possibly due to heterogeneity of diseases studied) most have commonly included alkylator based chemotherapy, often thiotepa or melphalan as well as ATG or radiation. Lang et al reported on 41 pediatric patients with both benign and malignant conditions who received a conditioning with fludarabine or clofarabine, thiotepa and melphalan in combination with either OKT3 or ATG-Fresenius. Five of the 41 patients did not engraft, yet all five successfully engrafted on second attempt after being reconditioned and infused with grafts from different haploidentical donors also depleted of αβ T cells and B cells. [49]

2.4.3. **Graft-versus-Host Disease (GVHD)**

So far, studies have shown promising results of TCR αβ/CD19+ depleted stem cell grafts for allogeneic HHCT with respect to the incidence of GVHD. Collectively, grade I-II aGVHD was reported in 3-39% of patients and primarily limited to the skin, rather than visceral organs. Across different studies, the overall incidence of severe GVHD was low (0-15%). Kaynar et al. studied 34 adults with AML or ALL who received a conditioning regimen of fludarabine, thiotepa, melphalan and ATG. Median follow-up was 176 days. Grade I-II aGVHD was observed in 26% of patients and grade III-IV aGVHD was observed in 6%. The rate of cGVHD was 6% with 3% being limited and 3% being extensive. The authors reported on at least two patients treated for GVHD using steroids, cyclosporine and mycophenolate. Locatelli et al. studied 80 pediatric patients with AML or ALL, each received one of four conditioning regimens, two of which were TBI-based. [50] There was no post transplantation GVHD prophylaxis. Median follow-up was 46 months. The rate of aGVHD was 30% and all were grade I-II. All of these patients responded to treatment with topical or systemic steroids. The rate of cGVHD was 5% with all cases limited to the skin. Bertaina el al. reported on 23 children with benign disorders who received one of four conditioning regimens. [34] There was no post-transplantation GVHD prophylaxis. Median follow-up was 18 months. Grade I-II aGVHD was observed in 23 patients and limited to the skin. No patients experienced acute visceral or cGVHD.

NOTE: see appendix B1 and B2 for grading systems

2.4.4. **Transplant-related Mortality (TRM)**

So far, studies have shown promising results of TCR αβ/CD19+ depleted stem cell grafts for allogeneic HHCT with respect to TRM. The reported incidence of TRM is variable and ranges from 0-20%. Infection was the most common cause of TRM. Lang et al. conducted a study involving 30 pediatric patients with non-malignant conditions, hematological malignancies and solid tumors who received a conditioning regimen of fludarabine, thiotepa and melphalan and either ATG or TBI. TRM in this study was 3.3%, with all TRM due to infection. [48] Bhat et al. carried out a study involving 22 pediatric patients with both malignant and non-malignant hematological conditions who received a conditioning regimen consisting of TBI or chemotherapy. TRM in this abstract was 14% with all TRM due to infection.[45] Overall the rate of TRM in these studies is lower than the rate of TRM seen in earlier studies involving transplantation of "megadoses" of CD34+ cells or CD3+/CD19+ depleted cells from haploidentical donors (36-40% and 20-30% respectively).[5,6,21,22,24]

2.4.5. **Survival**

Although survival data might be difficult to interpret from studies given the heterogeneity of diseases included, certain studies involving only one or two disease types provide insight regarding disease efficacy.

The study conducted by Locatelli et al. in 80 children with acute leukemia who received myeloablative conditioning, including ATG, showed OS of 72% at 5 years and DFS of 71% at 5 years.[50] Maschan et al. utilized this transplant method in 33 pediatric patients with high-risk AML with 20 receiving MUD transplants and 13 receiving haploidentical transplants. The conditioning regimen consisted of treosulfan, melphalan, fludarabine and ATG. OS and DFS at 2 years were 73% and 59% respectively for haploidentical cohort. [47]

2.4.6. **Immune Reconstitution**

Generally, studies evaluating immune reconstitution following HHCT with TCR αβ/CD19+ depleted grafts show rapid early reconstitution of γδ T cells and NK cells, with more gradual appearance of αβ T cells and B cells over 3-12 months.[33,45,48,50,53] Locatelli and colleagues demonstrated rapid recovery of γδ T cells and NK cells with a median of 181 cells/μL (range 1-1335) and 236 cells/μL (range 47-1813) at one month respectively.[50] Subsequently, αβ reconstitution took place, with a median of 47/μL (range 1-672) at 1 month and 186/μL (range 12-1340) at 3 months, but with recovery to 573/μL (range 135-2146) at 6 months and 1291/μL (range 259-2795) at 12 months. Similarly, B cell population had a very gradual recovery with minimal amounts detected at 1 and 3 months; ultimately, numbers reached 160/μL (range 2-1609) and 291/μL (range 40-1616) at 6 and 12 months respectively.[50] Similar results were previously reported by Lang and colleagues in pediatric patients where recovery of γδ T cells started as early as seven days post-transplant and made up the majority of CD3+ cells in the early post-transplant period. NK cells that were co-infused with the product were detected in the first week post-transplant followed by early

proliferation in week 2. NK cell population reached > 200 cells/μL shortly thereafter (with a median of 12.5 days). B cell population recovered by day 30 and reached normal levels by day 150 post-transplant.[49] Other advantages of the kinetics of NK cell recovery with HHCT using TCR αβ/CD19+ depleted grafted over CD34+ selection approach is that early donor derived mature NK cells is often not demonstrated in the CD34+ setting until at least 2-3 months post-transplant. [54] Airoldi and colleagues performed an in-depth analysis of γδ T cells recovery following TCR αβ/CD19+ depletion in 27 pediatric patients with malignant and non-malignant conditions. Two to three weeks post-transplant, T cell populations were predominantly γδ cells (mean 91.5% of CD3+ lymphocytes; range 70-100%) and with the αβ T cell population gradually increasing over time. Based on previous studies showing that aminobisphosphonates can induce activation and proliferation of Vδ2 subset of γδ population, the authors studied the effect of in vitro exposure to zoledronic acid on rate of expansion of γδ cells in subset of 13 patients. As expected, exposure to zoledronic acid increased expansion rate of Vδ2 subset of γδ cells. Similarly, cytotoxic activity of Vδ2 cells was enhanced with exposure of leukemia cell lines to zoledronic acid.[33]

2.4.7. **Infections**

CMV reactivation rates ranged from 30-70% in various studies. However, death from CMV occurred in no more than 5%. Two studies reported on BK virus reactivation, ranging from 17 to 25% in their cohorts, but no BK virus-related deaths were reported. Three studies report on adenovirus detection, ranging from 5-53%, with two deaths reported. [24,45,48] There were no cases of Epstein-Barr virus (EBV)-associated PTLD reported in the five studies where it was monitored, and EBV viremia was only very rarely detected. Overall, these data indicate that the risk of viral reactivations remains significant in the context of TCR αβ/CD19+ depleted HHCT. However, the reported mortality rates associated with these infections are very low. Importantly, risk of EBV reactivation and EBV-related PTLD appear very low in patients undergoing this type of transplant.

NOTE: See appendix C for CMV monitoring and prophylaxis

3.0 PATIENT ELIGIBILITY

3.1. Inclusion Criteria

3.1.1. Age less than or equal to 55 years

3.1.2. Lack of suitable conventional donor (10/10 HLA matched related or unrelated donor) or presence of rapidly progressive disease not permitting time to identify an HLA-matched unrelated donor. This does not include cord blood unit (CBU) availability.

3.1.3. Lansky/Karnofsky score > 50

3.1.4. Signed written informed consent

3.1.5. Diagnosis of one of the following:

a. Patient with life threatening hematological malignancy including “high-risk” ALL in first complete remission (CR1); ALL in second or subsequent remission (≥ CR2); high-risk AML in CR1; AML in second or subsequent CR; myelodysplastic syndromes (MDS); non-Hodgkin’s lymphomas (NHL) in second or subsequent remission (≥ CR2); CML
b. Hemophagocytic Lymphohistiocytosis (HLH) including familial HLH, relapsed HLH or central nervous system (CNS) HLH
c. Primary Immunodeficiency Disorders (PID)
d. Hemoglobinopathies including thalassemia or sickle cell disease (SCD)
e. Severe aplastic anemia (SAA) not responding to immune suppressive therapy
f. Congenital/hereditary cytopenias including Fanconi anemia (FA) without malignant clonal evolution (MDS, AML)
g. Other inherited bone marrow failure syndromes (IBMFS)
h. Severe chronic active Epstein Barr virus infection (SCAEBV) with predilection for T- or NK-cell malignancy

Note: ‘High risk’ ALL or AML refers to those acute leukemias identified by the presence of specific biologic features, which predict high likelihood of failure to conventional chemotherapy. As biologic features of high-risk disease evolve with improvement of conventional chemotherapy, it is not practical to define this indication with any further specificity. Therefore, high risk AML/ALL will be determined by the primary physician.

3.2 Exclusion Criteria

3.2.1 Life expectancy of $\underline{<}$ 6 weeks
3.2.2 Greater than grade II acute graft versus host disease (GVHD) or chronic extensive GVHD due to a previous allograft at the time of inclusion
3.2.3 Subject receiving an immunosuppressive treatment for GVHD treatment due to a previous allograft at the time of inclusion
3.2.4 Symptomatic cardiac disease or left ventricular shortening fraction < 25% or ejection fraction < 40%
3.2.5 Severe renal disease, with creatinine clearance < 40cc/1.73 m^2
3.2.6 Pre-existing severe restrictive pulmonary disease, FVC < 40% of predicted
3.2.7 Severe Hepatic Disease with ALT/AST ≥ x2.5 upper limit of normal or bilirubin level ≥ x1.5 upper limit of normal
3.2.8 Serious concurrent uncontrolled medical disorder or mental illness
3.2.9 Pregnant or breastfeeding female subject
3.2.10 Current active infectious disease including viral and fungal diseases at the time of enrollment; that on evaluation of PI precludes ablative chemotherapy or successful transplantation.
3.2.11 Active HIV infection
3.2.12 Severe personality disorder or mental illness that would preclude compliance with the study.

4.0 TREATMENT PLAN

We will use the CliniMACS® TCRαβ-Biotin system, manufactured by Miltenyi Biotech. This system will be accessed through three IDE exemption cross references: BB MF 12011, (Clinimacs® CD19 reagent system), BB MF 12251, (Clinimacs® depletion tubing set), and BB MF 15678, Clinimacs® TCR α/β reagent system), all held by the manufacturer, Milteny Biotec, Inc., Cambridge, MA.

4.1. Pretreatment Procedures

4.1.1. Patients will have a double lumen central venous catheter placed prior to starting conditioning regimen.

4.1.2. Patients will undergo pre-transplant workup which includes but not limited to disease evaluation, organ functioning, infectious disease workup (lab testing and radiologic exams) as well as performance scores. This will be performed per our institutional standards of practice.

4.2. Donor Selection

As described in section 3.1.2, the protocol will be open for patients who lack a suitable conventional donor or in the presence of a rapidly progressive disease not permitting time to identify an unrelated donor. For this protocol, the "best" donor will be defined as a first-degree haploidentical family member. Matching will be determined by class I and class II DNA based HLA typing. The donor should be sufficiently healthy not to be at increased risk from the mobilization procedure. Should more than one "equally" MHC incompatible donor be identified, other selection criteria will include: age and size of donor, CMV status and sex. The final decision on donor selection will be at the discretion of the primary BMT physician and the Principal Investigator (PI) of the study.

4.3. Donor Collections by Apheresis

Donor collection will be accomplished by using leukapheresis whereby the leukocyte fraction will be collected using continuous flow centrifugation. Prior to collection, infectious disease evaluation will be performed per institutional guidelines and standard of practice. The donor will receive recombinant G-CSF (Filgrastim) by daily subcutaneous injections (as per institutional SOP), typically 5 days prior to anticipated date of collection, with monitoring of CD34+ cell count starting on the fourth day of starting G-CSF. Apheresis will typically begin on the fifth day of mobilization. A standard apheresis procedure of 2-3 times blood volumes will be performed and processed per institutional practices. The volume processed and the duration of the apheresis will be documented and recorded. If less than 5 $x10^9$ peripheral blood mononuclear cells (PBMCs) are collected, the PI or designee should be notified immediately.

Every effort will be made to infuse a fresh stem cell product; however, a frozen product may be infused when necessary.

All donors will be monitored and PBSC products will be collected according to FACT-JACIE international standards for cell therapy, product collections, product and administration.

4.4. Donor Stem Cell Apheresis Procedure

Prior to negative depletion of TCR αβ+/CD19+, the target number of CD34+ stem cells by apheresis should be ≥ 12-15 $x10^6$/kg recipient weight. Accounting for up to 40% loss of CD34+ cells during the depletion process, the final product to be infused should contain at least 8-10 $x10^6$ CD34+ stem cells/kg recipient weight. The actual cell numbers achieved will be documented. To ensure achieving the target cell dose, a second successive day of apheresis will be allowed. The optimal number of peripheral CD34+ cells is ≥ 0.04 $x10^9$/L.

In the event that the CD34+ target cell dose is not achieved with the initial leukapheresis collection and, as stated above, the donor is to be collected again for additional CD34+ cells (with or without use of plerixafor).

4.5. TCR αβ+/CD19+ Depletion

The leukapheresis product will undergo negative selection of TCR αβ T cells following the standardized protocol in the user's manual for the CliniMACS (Miltenyi Biotech, Germany). TCR αβ+ T-cells are labeled by CliniMACS TCR αβ-Biotin (murine anti-TCR αβ monoclonal antibodies conjugated to biotin) which allows the TCR αβ+ T-cells to be magnetically labeled with CliniMACS Anti-Biotin reagents (murine anti-biotin monoclonal antibodies conjugated to superparamagnetic iron dextran particles) for depletion. The CD19+ B cells are labeled by CliniMACS CD19 reagents which allows the CD19+ B cells to be magnetically labeled for depletion. All unlabeled cells are selected as target cells which should not contain more than the maximum number of TCR αβ+.

The end points to be achieved for validation of the final product after negative selection include: 1) > 80% recovery of CD34+ cells after processing, 2) > 3-log depletion of TCR αβ cells after processing with a target infusion of < 1 $x10^5$/kg, 3) no growth in sterility cultures over 14 days and 4) > 80% viability in CD34+ cells. If at the end of the negative selection procedure, the residual number of TCR αβ+ cells is > 1 $x10^5$/kg, a residual part of the selected graft may be frozen and stored. The number of residual B lymphocytes after graft manipulation will be recorded but it will not lead to further graft manipulation or influence clinic decision.

If the desired CD34+ cell dose is not obtained in the initial product, a second leukapheresis collection may be selected for CD34+ cells after the target residual αβ T cell number has been reached. The number of residual B lymphocytes after the graft manipulation will be recorded but will not influence further graft manipulation or clinical decisions.

All subjects enrolled on the study will receive one dose of Rituximab i.v. at a dose of 200 mg/m2 once on day -1 regardless of the residual number of CD19 B cells in the final product.

4.6. Conditioning Regimens

Two types of regimens are allowed on the study, myeloablative and reduced intensity. Choice of regimen will be made at the discretion of the treating physician, that is deemed the most appropriate for each patient and for the condition/disease being treated.

NOTE:

- Target busulfan area under the curve (AUC) will be between 800-1,200 uM min.
- Dosing of chemotherapy for obese patients will follow the American Society for Blood and Marrow Transplantation Practice guideline Committee as described in the position statement published by Bubalo et al. in 2014 [55].

5.0 EVALUATIONS

5.1. Evaluation During Study

Study evaluations and their specific time points for each are described in details in the study calendar; will vary according to the underlying disease and will be performed per institutional standard of care.

Data will be collected as per secondary and exploratory objectives (section 1.1).

STUDY CALENDAR:

	Baseline Evaluations (prior to initiation of conditioning regimen)	Evaluations during inpatient admission	Weekly until Day +100	Day 100 Evaluations	Monthly through Month 11	Annual Evaluations Year 1 – 5
H & P	X@	See footnote 2	X	X	X	X
Body Weight	X		X	X	X	X
Performance Score	X	Day +30, Day +60		X		X
Chest X-ray or CT scan*	X					
EKG	X			X		X
ECHO	X			X		X
PFTs**	X			X		X
crCl or GFR***	X			X		
CBC with diff	X	See footnote 2	X	X	X	X
CMP including LDH	X		X	X	X	X
Liver panel	X		X	X	X	X
UA	X		X	X	X	X
HLA typing	X					
ABO typing	X			X		
Viral Serologies#	X					
Other Serologies#	X					
Viral PCR (plasma) including CMV, EBV and adenovirus PCR		See footnote 2	X	X	X	X (year 1 only)
Aspergillus Antigen			X	X	X	
Fungitell			X	X	X	
STR or FISH for sex chromosomes (if donor and recipient opposite sex) PB &	X	X (every 4 weeks following engraftment)		X	X	X
GVHD grading		X§	X	X	X	X

	Baseline Evaluations (prior to initiation of conditioning regimen)	Evaluations during inpatient admission	Weekly until Day +100	Day 100 Evaluations	Monthly through Month 11	Annual Evaluations Year 1 – 5
Bone marrow aspirate & Biopsy[%]	X	X (Day +28 +/- 3 days)		X	month 6 (as indicated)	X (Day +365 only +/- 3 days)
LP[%]	X	X (Day +28 +/- 3 days)		X		X (Day +365 only +/- 3 days)
Serum immunoglolin G			X	X	X	X
Restaging evaluations (as indicated)[^]	X			X		X (year 1 only)
Thyroid Function Tests						X
Quantitative serum immunoglobulins				X		X
T-, B- and NK-cell subset analysis [a]	See specific time points below (starting at Day +30)					
RTEs [b]						
T-cell Repertoire (flow cytometry) [c]						
Proportion of alpha/beta versus gamma/delta T-cell receptor [d]						
NK Screening Subset [e]						
Extended NK cell Phenotype [f]						

H&P: history and physical; EKG: electrocardiogram; ECHO: echocardiogram: PFTs: pulmonary function tests; CrCl: creatinine clearance; GFR: glomerular filtration rate; CMP: complete metabolic profile; UA: urinalysis; STR: short tandem repeats; FISH: fluorescent in-situ hybridization; PB: peripheral blood; LP: lumbar puncture; RTE: recent thymic emigrants

NOTE:

1) Other disease specific studies will be determined by the treating physician and will be considered standard of care clinical evaluations and not research-based studies.
2) Evaluations during inpatient admission will be performed per institutional standard of practice.

@ complete history and physical including:

- donor/recipient HLA matching Data
- underlying disease with number of remissions and relapses including cytoreductive therapy (as applicable)
- concurrent diseases or therapies

* CT scan to be done in place of chest X-ray if concern for possible ongoing pulmonary disease or infection (particularly fungal infection) or history of the above. Repeat imaging to be done as clinically indicated.

** May omit in young patient unable to perform testing. Recommended for those ≥ 6 years.

*** nuclear medicine GFR to replace CrCl (24 urine collection) is history of renal disease or if CrCl less than normal range for age

& Engraftment/Chimerism studies including STR and/or FISH to be done every 4 weeks until Day +100 following engraftment but no sooner than Day +28.

Serologies include hepatitis B, hepatitis C antibodies, HIV1/2, HSV, VZV, CMV, EBV, Toxoplasma and RPR. Sent to Gulf Coast laboratories

§ weekly evaluation to begin immediately following engraftment (see section 6.1 for definition)

% for patients with hematological malignancies, bone marrow aspirate and biopsy and lumbar puncture (as indicated) to be done at baseline, around day +28, day +100, day +180 and day +365 (+/- 3 days) post-transplant or as clinically indicated. Bone marrow evaluation to include morphology, flow cytometry (or other molecular based disease evaluation), Cytogenetics, FISH (as indicated) as well as other genomic molecular testing (as indicated).

^ restaging evaluations include PET-CT scan or other imaging modality for restaging or malignant disease as indicated

a) T-, B- and NK-cell subset analysis at Day 30, 60, 100, 180 and 365 post-HCT
b) Recent Thymic Emigrants (RTE) at Day 100, provided absolute lymphocytes count (ALC) is > 200 x10e9/L, then at Day 180 and 365
c) T-cell repertoire analysis by flow cytometry at Day 180 post-transplant
d) Proportion of alpha/beta versus gamma/delta T-cell receptor cell recovery at Day 30, 60, 100, 180 and 365 post-HCT
e) NK Screening subset at Day 30, 60, 100, 180 and 365 post-HCT
f) Extended NK cell phenotype at Day 30, 60, 100, 180 and 365 post-HCT

6.0 CRITERIA FOR STUDY EVALUATION

6.1. Hematopoietic engraftment: Engraftment will be recorded as the first of 3 consecutive days where absolute neutrophil count exceeds 0.5 $x10^9$/L
6.2. Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. http://ctep.cancer.gov
6.3. Patients will also be assessed post-transplant at 3, and 12 months by use of the Lansky or Karnofsky Performance Scale (see Appendix A)
6.4. Acute GVHD and chronic GVHD will be graded according to consensus criteria
6.5. Relapse will be assessed from regular blood and marrow analyses and will be recorded by the day of detection
6.6. Survival will be recorded by the day of death and the cause of death
6.7. Immune reconstitution will be determined from total lymphocyte count, from T and B cell numbers and from T cell subset analyses. Phenotyping will be done at approximately day +30, +60, +100, +180 and 365 post-HCT (+/- 7 days). We will also seek evidence of CMV, EBV and adenovirus reactivation, and will document all bacterial, viral and fungal infections.

7.0 SAMPLE SIZE CONSIDERATIONS

7.1. study design and sample size

This is a single site and single arm study to evaluate the safety and feasibility of αβ+ T cell-depleted haploidentical hematopoietic cell transplantation by negative selection of TCR αβ+ and CD19+ cells using automated CliniMACS device in patients with hematological malignancies and non-malignant disorders.

The primary safety endpoints are grade III or higher aGVHD by day 100 post HCT. The primary efficacy endpoint is a composite endpoint of both neutrophil and platelet engraftment by day 42 post HCT, with neutrophil engraftment defined as the first of 3

consecutive days with a peripheral blood absolute neutrophil count of ≥ $0.5x10^9$/L and platelet engraftment defined as the first day with platelet count of ≥ 20 $x10^9$/L without transfusion support for 7 consecutive days. Any eligible patients who receive HCT will be considered evaluable for both endpoints.

Based on previous experiences, we expect the engraftment rate is at least 85% and the aGVHD ≥ grade 3 rate is at most 10%. For the purpose of this protocol, the treatment regimen is deemed useless if the engraftment rate is only 70% or the aGVHD ≥ grade 3 rate is more than 30%. A Bryant and Day[56] two-stage optimal design will be used in order to allow early stopping for lack of satisfactory or excessive aGVHD. Although GVHD only occurs after engraftment, we anticipate all patients will achieve engraftment so the GVHD rate among engrafted patients after HCT will be very similar with that among patients who receive HCT. Therefore, an acceptable aGVHD rate of 10% and an unacceptable rate of 30% are still used for the Bryant and Day design. The study will enroll a total of 47 (18+29) evaluable patients in two stages, with a one-sided significance level of 10% for efficacy, a one-sided significance level of 5% for toxicity, and 80% Power. This design results in an expected maximum sample size of 28 if engraftment rate and/or aGVHD rate are unacceptable. The probability of stopping early due to low engraftment and excessive aGVHD is 89%; due to excessive aGVHD, but with acceptable engraftment rate is 71%; and due to low engraftment rate with acceptable aGVHD rate is 68%.

7.2. Stopping Rules

Based on the Bryant and Day two-stage design, at the end of the first stage, if the number of engraftment is less than or equal to 13 or if the number of aGVHD ≥ grade 3 is more than or equal to 5 out of 18 patients, the study will be stopped early due to either low efficacy or high toxicity. If we observe an engraftment rate <=90% after the first stage, we will report the aGVHD rate among the engrafted patients and consult with the FDA. Otherwise if not stopping at the first stage, the study proceeds to the second stage and enrolls 29 more patients. At the end of study, if 36 or fewer patients have engrafted or 10 or more patients experience aGVHD ≥ grade 3, the treatment regimen will be considered to demonstrate low efficacy or high toxicity.

Additional accrual halting rules for the study to mitigate risks for patients will be applied in any of the following settings:

- any treatment-related death within 100 days of HSCT, not related to disease progression;
- occurrence of any case of post transplantation lymphoproliferative disorder (PTLD);
- excessive incidence of failure to engraftment is ≥30% when the number of patients who failed engraftment is equal or greater than the boundary shown in the table below;
- excessive incidence of grade 3 or higher acute or chronic GVHD is ≥30% when the number of patients who experience grade 3 or higher acute or chronic GVHD is equal or greater than the boundary shown in the table below.

Number of treated patients (n)	6	12	24	30	36	42
Boundary (x)	2	4	8	9	11	13

Once halted, the study team will review the outcomes of the patients and report to regulatory authorities for review. The study will not resume until approvals to continue are granted by the FDA.

7.3. Statistical Analysis

Incidences of neutrophil and platelet engraftment by day 42 post HCT from enrolled evaluable patients will be estimated along with the associated 95% confidence intervals.

Incidence of acute GVHD and chronic GVHD by grades among patients who achieve engraftment will be reported as rate and its associated 95% confidence interval. Other adverse events will also be summarized descriptively as frequency tables. Listings of all adverse events by patients will include the time to onset, the duration of each event, the severity of each event, and the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal.

Feasibility of the automated CliniMACS system to produce a graft with defined cell composition will be reported as proportion of patients with success in using CliniMACS® System for processing hematopoietic stem and progenitor cells collected from an allogeneic, HLA-haplotype donors to obtain a CD34+ cell-enriched population with the target dose of CD34+ cells of 10-12 $x10^6$/kg (minimum of ≥$2.5x10^6$/kg) and a target dose of T-cell receptor (TCR) alpha/beta CD3+ cells is ≤$1x10^5$/kg.

Cumulative incidence of transplant-related mortality (TRM) at Day 100 and Day 365 among all evaluable patients will be reported with frequencies and proportions. OS will be summarized with survival curves using Kaplan-Meier method. OS is defined as the time from HCT to death. Alive patients will be censored at the last follow-up.

Incidences of viral reactivations, defined as detectable viral load by peripheral blood PCR, and symptomatic infections up to one year post HCT will be summarized as proportions among all evaluable patients.

For patients with hematological malignancies, complete donor chimerism by day 30 post-transplant will be summarized descriptively. Lab data including lymphocyte, T cell, B cell, and NK cell at each time point post HSC will be summarized appropriately and presented in plots. Testing of trends over time will be performed with paired statistical methods but the analyses will be considered exploratory.

8.0 REPORTING REQUIREMENTS

8.1. To register patients, contact Dr. Erin Morales at 832-826-0860 at Texas Children's Hospital or Dr. George Carrum at 713-441-1450 at Houston Methodist Hospital. After entry, the following data will be collected:

8.1.1. Eligibility (at enrollment)
8.1.2. On study
8.1.3. Concomitant Medications
8.1.4. Adverse Events
8.1.5. Off study

8.1.6. Death information (if applicable)
8.1.7. Other transplant related data is collected on CIBMTR forms as required

8.2. Relapse and survival data will be collected until one year post-transplant

8.3. Drug Toxicity and/or Adverse Reactions

8.3.1. Adverse events will be collected as per SOP J02.05.XX and SOP J02.78.XX
8.3.2. Serious adverse events will be collected and reported as per SOP J02.06.XX which includes the requirement that serious adverse events, particularly Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the FDA per IND safety reporting requirement: 21 CFR 312.32

8.4. Potential Risks and Mitigation Strategies

Adverse events (including grade 3 or higher) are possible and expected toxicities associated with any transplant procedure regardless of the graft processing approach, donor type or whether transplant is autologous or allogeneic. These are primarily attributable to conditioning regimens including chemotherapy and/or radiation therapy, immunosuppressive medications (which will not be a standard of care on this protocol) as well as other supportive interventions (including antimicrobials, diuretics, among others). Accordingly, the occurrence of AEs (other than GVHD exceeding the acceptable rates specified in the study protocol) should not result in early termination of the study or pause in study accrual.

Management of potential post-transplant complications will be done per institutional guidelines and as outlined in the SOPs below:

- F05.07.XX Treatment of Regimen-related Complications
- F07.04.XX Treatment of Infections
- F08.03.XX Acute GVHD Treatment
- F08.04.XX Chronic GVHD Treatment
- F05.05.XX Adverse Reactions to Transplantation
- F05.02.XX Post-Transplant Evaluation
- F05.15.XX Recipient Care

Subjects with engraftment failure (as defined in the study protocol), will be taken off study, will be evaluated for a second allogeneic HCT using a standard of care approach and per our institutional guidelines.

9.0 OFF STUDY CRITERIA

- Refusal of further study follow-up by patient or legal guardian
- Treatment with hematopoietic cell product, relating to patients receiving additional stem and progenitor cell products
- Completion of study specified procedures
- Relapse
- Lost to follow-up
- Death

10.0 CLINICAL TRIAL OVERSIGHT AND MONITORING

This protocol will be conducted in accordance with the Cell/Gene Therapy Monitoring Plan on file with the FDA.

This protocol will be monitored in accordance with the current Data Safety Monitoring Plan of the Dan L Duncan Cancer Center at Baylor College of Medicine.

The conduct of this clinical trial will be evaluated in accordance with the Texas Children's Cancer Center and Center for Cell and Gene Therapy Quality Assurance Policy and Procedure Plan and Quality Control Policy and Procedure Plan.

11.0 INFORMED CONSENT

All patients and/or their legal guardian must sign a document of informed consent consistent with local institutional and federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted for treatment without such a document signed by the patient or their legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines.

Appendix A: Lansky and Karnofsky Performance Scale

Karnofsky Scale (recipient age ≥ 16 years)	Lansky Scale (recipient age ≥ 1 year and <16 years)
Able to carry on normal activity; no special care is needed	**Able to carry on normal activity; no special care is needed**
100 Normal, no complaints, no evidence of disease	**100** Fully active
90 Able to carry on normal activity	**90** Minor restriction in physically strenuous play
80 Normal activity with effort	**80** Restricted in strenuous play, tires more easily, otherwise active
Unable to work, able to live at home, cares for most personal needs, a varying amount of assistance is needed	**Mild to moderate restriction**
70 Cares for self, unable to carry on normal activity or to do active work	**70** Both greater restrictions of, and less time spent in active play
60 Requires occasional assistance but is able to care for most needs	**60** Ambulatory up to 50% of time, limited active play with assistance/supervision
50 Requires considerable assistance and frequent medical care	**50** Considerable assistance required for any active play, fully able to engage in quiet play
Unable to care for self, requires equivalent of institutional or hospital care, disease may be progressing rapidly	**Moderate to severe restriction**
40 Disabled, requires special care and assistance	**40** Able to initiate quite activities
30 Severely disabled, hospitalization indicated, although death not imminent	**30** Needs considerable assistance for quiet activity
20 Very sick, hospitalization necessary	**20** Limited to very passive activity initiated by others (e.g., TV)
10 Moribund, fatal process progressing rapidly	**10** Completely disabled, not even passive play

Appendix B1: Acute GVHD Staging and Grading (MAGIC Consortium)

Stage	Skin (active erythema only)	Liver (bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	< 2 mg/dl	No or intermittent nausea, vomiting or anorexia	Adult: < 500 ml/day or < 3 episodes/day Child: < 10 ml/kg/day or < 4 episodes/day
1	Maculopapular rash < 25% BSA	2-3 mg/dl	Persistent nausea, vomiting or anorexia	Adult: 500-999 ml/day or 3-4 episodes/day Child: 10-19.9 ml/kg/day or 4-6 episodes/day
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dl	-	Adult: 1000-1500 ml/day or 5-7 episodes/day Child: 20-30 ml/kg/day or 7-10 episodes/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dl	-	Adult: > 1500 ml/day or > 7 episodes/day Child: > 30 ml/kg/day or > 10 episodes/day
4	Generalized erythroderma (> 50% BSA) plus bullous formation and desquamation > 5% BSA	> 15 mg/dl	-	Severe abdominal pain with or without ileus, or grossly bloody stools (regardless of stool volume)

Overall clinical grade (based upon most severe target organ involvement):

Grade 0: No stage 1-4 of any organ

Grade I: Stage 1-2 skin without liver, upper GI or lower GI involvement

Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI

Grade III: stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI

Grade IV: stage 4 skin, liver or lower GI involvement, with stage 0-1 upper GI

Abbreviations: BSA = body surface area; GI = gastrointestinal

Appendix B2: Chronic GVHD Staging and Grading (NIH Consensus Grading)

Chronic GVHD will be assessed at least every 3 months using the NIH Consensus staging and grading (see table below from *Jagasia et al*). Every attempt to diagnose patients with physical criteria should be made. Histologic criteria should be used if needed.

Utilize chronic GVHD organ scoring system (0-3) from *Jagasia et al* on the following pages The organ and site-specific scores are then combined for a **global assessment of chronic GVHD severity** (mild, moderate, or severe).* (Table adapted from *Jagasia et al.*)

NIH Global Severity of Chronic GVHD

Mild chronic GVHD

1 or 2 organs involved with no more than score 1 *plus*
Lung score 0

Moderate chronic GVHD

3 or more organs involved with no more than score 1

OR

At least 1 organ (not lung) with a score of 2

OR

Lung score 1

Severe chronic GVHD

At least 1 organ with a score of 3

OR

Lung score of 2 or 3

Key points:

1 In skin: higher of the two scores to be used for calculating global severity.

2 In lung: FEV1 is used instead of clinical score for calculating global severity.

3 If the entire abnormality in an organ is noted to be unequivocally explained by a non-GVHD documented cause, that organ is not included for calculation of the global severity.

4 If the abnormality in an organ is attributed to multifactorial causes (GVHD plus other causes) the scored organ will be used for calculation of the global severity regardless of the contributing causes (no downgrading of organ severity score).

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† ☐ **SCORE % BSA** *GVHD features to be scored by BSA:* **Check all that apply:** Maculopapular rash/erythema; Lichen planus-like features; Sclerotic features; Papulosquamous lesions or ichthyosis; Keratosis pilaris-like GVHD	No BSA involved	1-18% BSA	19-50% BSA	>50% BSA
SKIN FEATURES SCORE:	No sclerotic features		**S**uperficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** Deep sclerotic features; "Hidebound" (unable to pinch); Impaired mobility; Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** Hyperpigmentation; Hypopigmentation; Poikiloderma; Severe or generalized pruritus; Hair involvement; Nail involvement; *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
MOUTH *Lichen planus-like features present:* **Yes** **No**	No symptoms	Mild symptoms **with** disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **with** partial limitation of oral intake	Severe symptoms with disease signs on examination **with** major limitation of oral intake
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* **Yes** **No** **Not examined**	No symptoms	Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GI Tract ***Check all that apply:*** Esophageal web/ proximal stricture or ring Dysphagia Anorexia Nausea Vomiting Diarrhea Weight loss ≥5%* Failure to thrive	No symptoms	Symptoms without significant weight loss* (<5%)	Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
LIVER	Normal total bilirubin and ALT or AP < 3 x ULN	Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	Elevated total bilirubin > 3 mg/dL
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
LUNGS**				
Symptom score:	No symptoms	Mild symptoms (shortness of breath after climbing one flight of steps)	Moderate symptoms (shortness of breath after walking on flat ground)	Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **%** FEV1 ☐	FEV1≥80%	FEV1 60-79%	FEV1 40-59%	FEV1 ≤39%
Pulmonary function tests Not performed				
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

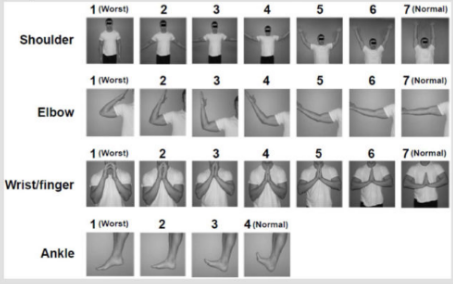
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	No symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) Not examined *Currently sexually active* Yes No	No signs	Mild signs‡ and females with or without discomfort on exam	Moderate signs‡ and may have symptoms with discomfort on exam	Severe signs‡ with or without symptoms
*Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

Ascites (serositis)___	Myasthenia Gravis___	
Pericardial Effusion___	Peripheral Neuropathy___	Eosinophilia > 500/µl___
Pleural Effusion(s)___	Polymyositis___	Platelets <100,000/µl ___
Nephrotic syndrome___	Weight loss>5%* without GI symptoms ___	Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ❑ **No GVHD** ❑ **Mild** ❑ **Moderate** ❑ **Severe**

Photographic Range of Motion (P-ROM)



† Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring.

* Weight loss within 3 months.

**Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

Abbreviations: ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); ULN (normal upper limit).

‡ To be completed by specialist or trained medical providers (see Supplemental Figure).

Figure 1.
Organ Scoring of Chronic GVHD

FORM A

Current Patient Weight: _____ Today's Date: _____ MR#/Name: _____

CHRONIC GVHD ACTIVITY ASSESSMENT- CLINICIAN

Health Care Provider Global Ratings:

0=none
1= mild
2=moderate
3=severe

Where would you rate the severity of this patient's chronic GvHD symptoms on the following scale, where 0 is cGVHD symptoms that are not at all severe and 10 is the most severe cGVHD symptoms possible:

0 1 2 3 4 5 6 7 8 9 10

cGvHD symptoms not at all severe — **Most severe cGvHD symptoms possible**

Over the <<time>> would you say that this patient's cGvHD is

+3= Very much better
+2= Moderately better
+1= A little better
0= About the same
-1=A little worse
-2=Moderately worse
-3=Very much worse

Mouth	**Erythema**	None	0	Mild erythema or moderate erythema (<25%)	1	Moderate (≥25%) or Severe erythema (<25%)	2	Severe erythema (≥25%)	3
	Lichenoid	None	0	Lichen-like changes (<25%)	1	Lichen-like changes (25-50%)	2	Lichen-like changes (>50%)	3
	Ulcers	None	0			Ulcers involving (≤20%)	3	Severe ulcerations (>20%)	6
				Total score for all mucosal changes					

Gastrointestinal-Esophageal • Dysphagia OR Odynophagia	*0= no esophageal symptoms* *1=Occasional dysphagia or odynophagia with solid food or pills during the past week* *2=Intermittent dysphagia or odynophagia with solid foods or pills, but not for liquids or soft foods, during the past week* *3=Dysphagia or odynophagia for almost all oral intake, on almost every day of the past week*
Gastrointestinal-Upper GI • Early satiety OR • Anorexia OR • Nausea & Vomiting	*0= no symptoms* *1=mild, occasional symptoms, with little reduction in oral intake during the past week* *2=moderate, intermittent symptoms, with some reduction in oral intake during the past week* *3=more severe or persistent symptoms throughout the day, with marked reduction in oral intake, on almost every day of the past week*
Gastrointestinal-Lower GI • Diarrhea	*0= no loose or liquid stools during the past week* *1= occasional loose or liquid stools, on some days during the past week* *2=intermittent loose or liquid stools throughout the day, on almost every day of the past week, **without requiring** intervention to prevent or correct volume depletion* *3=voluminous diarrhea on almost every day of the past week, **requiring** intervention to prevent or correct volume depletion*

Lungs (Liters and % predicted) • Bronchiolitis Obliterans	FEV1	FVC	Single Breath DLCO (adjusted for hemoglobin)		TLC	RV
Liver Values	Total serum bilirubin mg/dL	ULN mg/dL	ALT U/L	ULN U/L	Alkaline Phosphatase U/L	ULN U/L
Baseline Values	Total Distance Walked in 2 or 6 Mins: ❑ 2 min ❑ 6 min		Karnofsky or Lansky	Platelet Count K/uL	Total WBC K/uL	Eosinophils %

Abnormality present but explained entirely by non-GVHD documented cause (specify site/alternate cause):_____

Abnormality present but explained entirely by non-GVHD documented cause (specify site/alternate cause):_____

Abnormality present but explained entirely by non-GVHD documented cause (specify site/alternate cause):_____

*Adapted from Lee et al, 2015

FORM B

Today's Date: _____ MR#/Name: _____

CHRONIC GVHD ACTIVITY ASSESSMENT-PATIENT SELF REPORT

Symptoms **Please rate how severe the following symptoms have been in the last seven days. Please fill in the circle below from 0 (symptom has not been present) to 10 (the symptom was as bad as you can imagine it could be) for each item.**	**Not Present** 0	1	2	3	4	5	6	7	8	9	**As Bad As You Can Imagine** 10
Your **skin itching** at its WORST?	O	O	O	O	O	O	O	O	O	O	O
Your **skin and/or joint tightening** at their WORST?	O	O	O	O	O	O	O	O	O	O	O
Your **mouth sensitivity** at its WORST?	O	O	O	O	O	O	O	O	O	O	O
Your **genital discomfort** at its WORST? (Women – vagina, vulva, or labia) (Men – penis)	O	O	O	O	O	O	O	O	O	O	O

Eyes	What is your main complaint with regard to your eyes?	
	Please rate how severe this symptom is, from 0 (not at all severe) to 10 (most severe):	0 1 2 3 4 5 6 7 8 9 10

Patient Global Ratings:

1. Overall, do you think that your chronic graft versus host disease is mild, moderate or severe?
1= mild
2=moderate
3=severe

2. Please circle the number indicating how severe your chronic graft versus host disease symptoms are, where 0 is cGvHD symptoms that are not at all severe and 10 is the most severe chronic GvHD symptoms possible.

0 1 2 3 4 5 6 7 8 9 10

cGvHD symptoms not at all severe — Most severe cGvHD symptoms possible

3. Compared to a month ago, overall would you say that your cGvHD symptoms are:

+3= Very much better
+2= Moderately better
+1=A little better
0= About the same
-1=A little worse
-2=Moderately worse
-3=Very much worse

*Adapted from Lee et al, 2015

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Appendix C: CMV monitoring and Prophylaxis:

CMV prophylaxis (CMV seropositive recipients) with letermovir 480 mg daily beginning between day 0 and day 28; to continue until at least day 100; if letermovir is not approved for use in age range, consider on an individual basis after consultation with pharmacist and infectious diseases.

OR

For CMV-seronegative recipients receiving CMV-seropositive donor product can consider prophylaxis with ganciclovir or valganciclovir (BID for 2 weeks, then daily Monday-Friday) from engraftment to at least day 100.

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