CITY OF HOPE
1500 E. DUARTE ROAD
DUARTE, CA 91010

DEPARTMENT OF HEMATOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: A PILOT STUDY OF POST-TRANSPLANT HIGH DOSE CYCLOPHOSPHAMIDE (PTCY) AS PART OF GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS IN T-CELL REPLETE HLA-MISMATCHED UNRELATED DONOR (MMUD) ABLATIVE AND REDUCED INTENSITY HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR HEMATOLOGICAL MALIGNANCIES

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 16419 **PROTOCOL DATE: 07/03/2019**

Initial Submission	Protocol dated 05/22/2017	Version: 00
COH Amendment 01	Protocol dated 06/13/2017	Version: 01
COH Amendment 02	Protocol dated 12/08/2017	Version: 02
COH Amendment 03	Title Page dated 03/11/2019	Version: 03
COH Amendment 04	Protocol dated 07/03/2019	Version: 04
COH Amendment 05	Protocol dated 07/03/2019 TP	Packet: 05

SITE: Bone Marrow and Extramedullary
STAGE (If applicable): N/A
MODALITY: IV
TYPE: Pilot

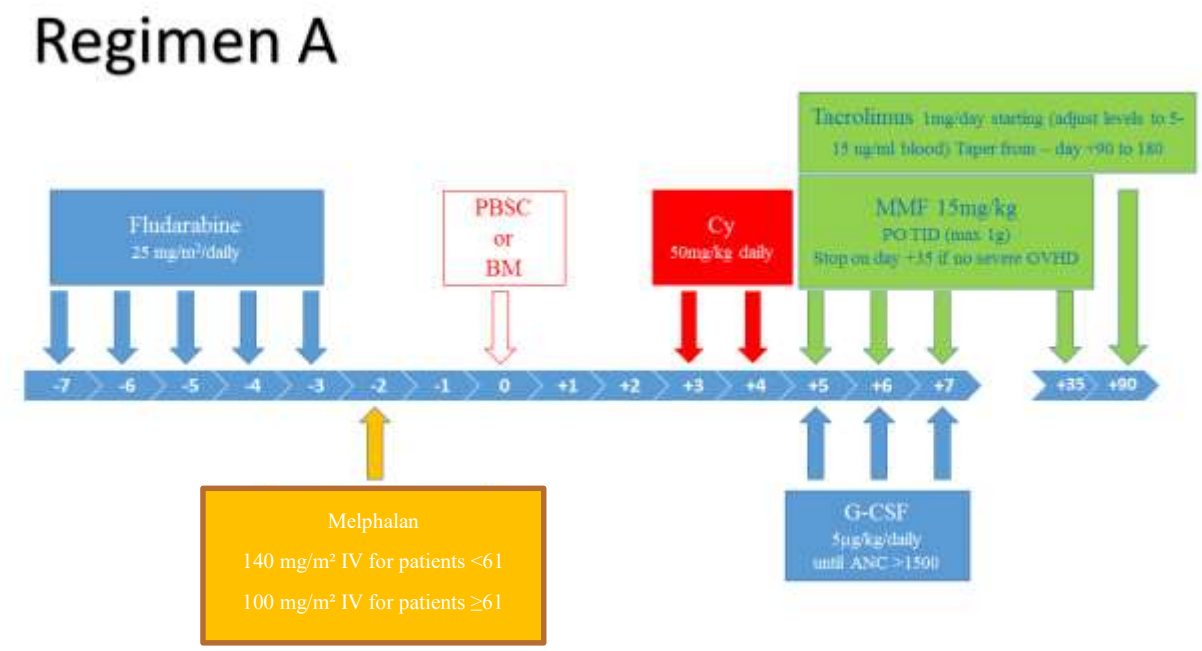
PRINCIPAL INVESTIGATOR: Monzr Al Malki, M.D.

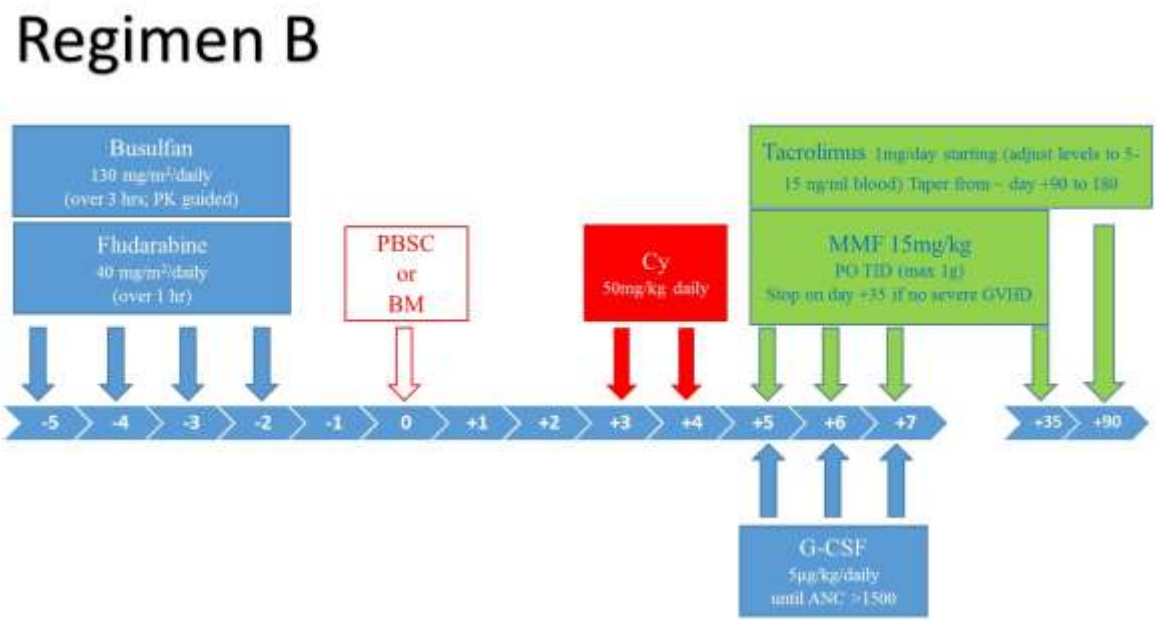
PARTICIPATING CLINICIANS: Haris Ali, M.D., Ibrahim Aldoss, M.D., L. Elizabeth Budde, M.D., Ji-Lian Cai, M.D., Thai Cao, M.D., Leonardo Farol, M.D., Stephen Forman, M.D., Alex Herrera, M.D., Myo Htut, M.D., Chatchada Karanes, M.D., Samer Khaled, M.D., Amrita Krishnan, M.D., Guido Marcucci, M.D., Matthew Mei, M.D., Ryotaro Nakamura, M.D., Nitya Nathwani, M.D., Anna Pawlowska, M.D, Leslie Popplewell, M.D., Vinod Pullarkat, M.D., Joseph Rosenthal, M.D., Michael Rosenzweig, M.D., Firoozeh Sahebi, M.D., Eileen Smith, M.D., Amandeep Salhotra, M.D., Tanya Siddiqi, M.D., Anthony Stein, M.D., David Snyder, M.D., Ricardo Spielberger, M.D., Jeffrey Wong, M.D., Jasmine Zain, M.D., Karamjeet Sandhu, M.D., Liana Nikolaenko, M.D., Eric Radany, M.D, Nicole Karras, M.D.

BIOSTATISTICIAN: Ni-Chun Tsai

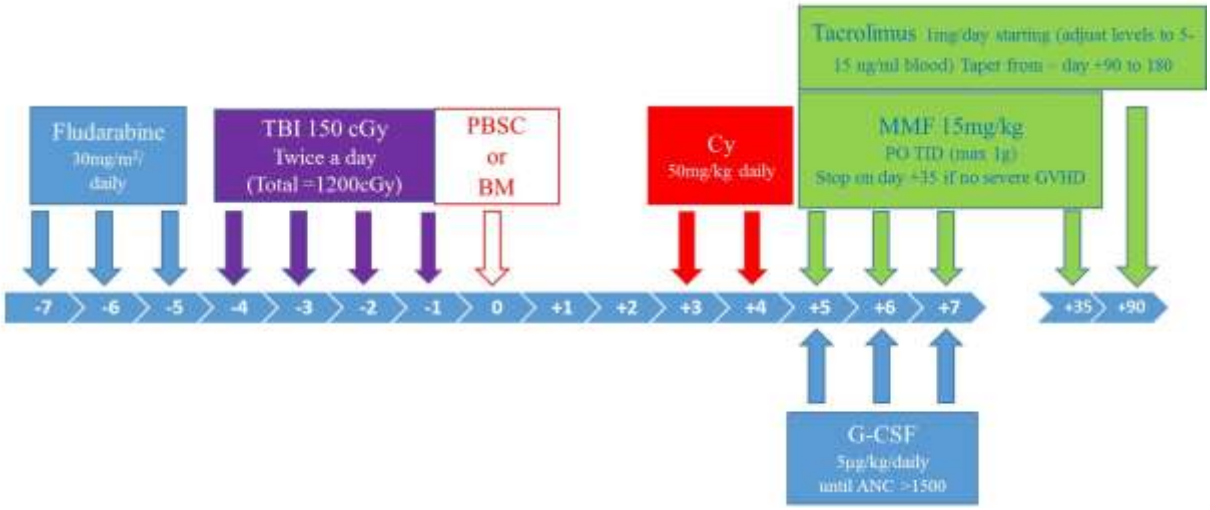
Experimental Design Schemas

Note: A window of 1-2 days is allowed for stem cell availability.





Regimen C


TacroImus 1mg/day starting (adjust levels to 5-15 ug/ml blood) Taper from – day +90 to 180
Fludarabine
30mg/m²/ daily
TBI 150 cGy
Twice a day
(Total =1200cGy)
PBSC
or
BM
Cy
50mg/kg daily
MMF 15mg/kg
PO TID (max 1g)
Stop on day +35 if no severe GVHD
-7
-6
-5
-4
-3
-2
-1
0
+1
+2
+3
+4
+5
+6
+7
+35
+90
G-CSF
5µg/kg/daily
until ANC >1500

Protocol Synopsis

Protocol Title:
A PILOT STUDY OF POST-TRANSPLANT HIGH DOSE CYCLOPHOSPHAMIDE (PTCY) AS PART OF GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS IN T-CELL REPLETE HLA-MISMATCHED UNRELATED DONOR (MMUD) ABLATIVE AND REDUCED INTENSITY HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR HEMATOLOGICAL MALIGNANCIES
Brief Protocol Title for the Lay Public (if applicable):
Use of Cyclophosphamide for Graft-versus-Host Disease in mismatched unrelated donor HCT.
Study Phase:
Pilot
Participating Sites:
Single Center: City of Hope National Medical Center
Rationale for this Study:
MMUD HCT is associated with poorer outcome primarily due to increased risk of GVHD-related mortality. At City of Hope, we have explored and evaluated the use of Tacrolimus/Sirolimus-based GVHD prophylaxis for MUD and MMUD HCT over the past 10 years. While overall results have been satisfactory in MUD HCT, the outcome of MMUD HCT was inferior to MUD HCT using Tacrolimus/Sirolimus-based regimens. PTCy has been shown to be effective in preventing GVHD in haploidentical donor HCT and MUD HCT. However, limited data exist for its efficacy in preventing GVHD and improving outcomes of MMUD HCT. In this protocol, we aim to investigate the ability of PTCy to overcome the increased incidence of GVHD and subsequent increased risk of non-relapse mortality (NRM) expected after MMUD HCT.
Objectives:
In patients undergoing T-cell replete HLA-mismatched unrelated donor hematopoietic cell transplantation (HCT) for hematologic malignancy, using either an ablative or reduced intensity conditioning regimen: Primary objective: • To estimate the GVHD-free relapse/progression-free survival (GRFS) at one-year post HCT and to evaluate the clinical activity of post-transplant high dose cyclophosphamide (PTCy). Secondary objectives: ▪ To summarize toxicities/complications/infections including type, frequency, severity, attribution, time course and duration through 100 days post-transplant. ▪ To estimate the cumulative incidence (CI) of acute and chronic GVHD. ▪ To characterize the time course of neutrophil and platelet recovery/engraftment. ▪ To estimate overall survival (OS), progression-free survival (PFS), CI of relapse/progression, and non-relapse mortality (NRM) at 100 days, 1 year and 2 years. ▪ To describe quality of life at 100 days, 6 months, 1 and 2 years. ▪ To characterize immune cell reconstitution and T cell repertoire after MMUD HCT with

PTCy.

- To characterize quality of life.

Study Design:

Pilot/Estimation Design, Single Center
[Estimation: GVHD-free relapse/progression-free survival (GRFS) at one-year post HCT in Ablative and Non-Myeloablative strata.]

Endpoints:

Primary Endpoint:

- GVHD-free relapse/progression-free survival (GRFS) is defined as time from start of treatment (HCT) to grade 3-4 acute GVHD or moderate-severe chronic GVHD, relapse, progression, or death (from any cause), whichever occurs first.

Secondary Endpoints:

- Toxicity/infections
- Acute and Chronic GVHD
- Engraftment (Neutrophil and Platelet Recovery)
- Overall and Progression-Free Survival
- Relapse/Progression
- Quality of Life using validated tools

Sample Size:

The target sample size is 38 patients accrued into two strata: 19 patients will receive myeloablative conditioning (MAC: Regimens B or C) and 19 will receive reduced intensity conditioning (RIC: Regimen A).

Estimated Duration of the Study

Subject accrual: 24 Months

Follow-up Period**:** 24 Months

Summary of Subject Eligibility Criteria:

Inclusion Criteria:

- Patients with neoplastic hematological disorders with an indication for allogeneic transplant according to the standard guidelines as follows:
  - Acute leukemia [AL] in CR1 or subsequent CR or active disease with BM blasts of <10%.
  - Chemosensitive Hodgkin, Non-Hodgkin lymphoma [HL or NHL] or chronic lymphocytic leukemia [CLL]: high risk upfront with 17q-, relapsed less than 1 year after chemotherapy, relapse after previous autologous transplant, or failure to achieve CR with chemotherapy. All other types of lymphoma are eligible.
  - Chronic myeloid leukemia [CML] in hematological remission after blast/accelerated phase or chronic phase refractory to multiple TKI.
  - Myelodysplastic syndrome [MDS] with intermediate-2 or high risk per IPSS or myeloproliferative neoplasm; primary or secondary if high-risk features or refractory

disease.
    - High risk, or refractory and relapsed multiple myeloma [MM].
- No available suitable HLA-matched donor.
- Age Criteria: 5 to 75 years of age if meets criteria for HCT per SOP.
- Organ Function Criteria: The following organ function testing should be done within 30 days before study registration. In case of active disease evaluation should be within 15 days.
    - Cardiac: LVEF of 50% or above, by MUGA or Echocardiogram.
    - Pulmonary: FVC, FEV1 and DLCO (corrected) should be ≥50% of expected.
    - Renal: serum creatinine level to be < 2 mg/dl or Measured creatinine clearance (CrCl) must be equal or greater than 60 mL/min. The updated Schwartz formula should be used for pediatric patients (≥5 to 12 years old).
    - Hepatic: serum bilirubin 1.5 upper limits of normal (ULN), (AST)/ (ALT) 2.5 ULN, and alkaline phosphatase 2.5 ULN.
- Performance status: Karnofsky ≥ 70%.
- Consent: All patients must be informed of the investigational nature of this study and given written informed consent in accordance with institutional and federal guidelines.

Exclusion Criteria:

- Psychosocial issues: no appropriate caregivers identified, or non-compliant to medications
- Uncontrolled medical or psychiatric disorders which may preclude patients to undergo clinical studies (Discretion of the attending physician)
- Active infection or second malignancy.
- HIV1 (Human Immunodeficiency Virus-1) or HIV2 positive.
- Patient with active Hepatitis B or C determined by polymerase chain reaction (PCR).
- Pregnant or breastfeeding.

Donor Eligibility Criteria: Donor choice per MUD committee.

- 7/8 with either antigen or allele mismatched HLA (-A, -B, -C, and -DR) or 8/8 HLA-matched with either double DQ mismatch (8/10 match) or combined DQ and DP mismatch (10/12 match).
- Suitable Donor – Medically cleared to donate per NMDP.
- Absence of donor-specific antibodies (DSA) to the mismatched HLA- locus.

Investigational Product Dosage and Administration:

The conditioning regimen will consist of either myeloablative (MAC) or reduced intensity conditioning (RIC):

Regimen A (RIC):

- Fludarabine 25 mg/m^2/day IV Days –7, –6, –5, –4, –3
- Melphalan 140 mg/m^2 IV Days -2 (or 100 mg/m^2 IV if age ≥60)
- Day 0 will be the day of infusion of T-cell replete PBSC or BM

Regimen B (MAC):

- Fludarabine 40 mg/m^2 over 1hr IV Days -5, -4, -3, -2
- Busulfan 130 mg/m^2 over 3 hrs. Days -5, -4, -3, -2 (PK-guided)
- Day 0 will be the day of infusion of T-cell replete PBSC or BM

Regimen C (MAC):

- Fludarabine 30 mg/m^2 IV Days -7, -6, -5
- TBI 150 cGy twice a day Days -4, -3, -2, -1 (total 1200 cGy)
- Day 0 will be the day of infusion of T-cell replete PBSC or BM

GVHD prophylaxis regimen

-Cy 50 mg/kg/day IV Days +3, +4 (per institutional preference)

-Tacrolimus 1 mg continuous IV beginning Day +5 with dose adjusted to maintain a level of 5-15 ng/mL will be changed to equivalent PO dose once stable. Taper starts around day 90+ if no active GVHD

-Mycophenolate mofetil (MMF) 15 mg/kg PO TID beginning Day +5, Maximum dose 1g PO TID. MMF will be stopped Day +35 if no severe GVHD

-G-CSF 5 µg/kg/day beginning Day +5 until ANC >1,500/mm3 for 3 consecutive days

Clinical Observations and Tests to be Performed:

Clinical observations include the endpoints;

- GRFS, defined as time from start of treatment (HCT) to grade 3-4 acute GVHD or moderate-severe chronic GVHD, relapse, progression or death, whichever occurs first.
- Toxicity/infections
- Acute and Chronic GVHD
- Engraftment (Neutrophil and Platelet Recovery)
- Overall and Progression-Free Survival
- Relapse/Progression
- Quality of Life using validated tools

Other routine evaluations and ancillary tests will be performed as per standard of care (SOC). As research correlative studies we will evaluate the reconstitution of the immune system including a comprehensive characterization of the T cell receptor (TCR) repertoire in patients who underwent MMUD HCT using a next generation sequencing (NSG) approach.

Statistical Considerations:

We will be examining the clinical activity and safety of PTCy in patients undergoing T-cell replete MMUD HCT for hematologic malignancy. A total of 38 patients, 19 patients per stratum is sufficient to estimate the GRFS rate at 1 year with adequate precision (standard error = .08 overall, = 0.12 per stratum).

- Survival estimates will be calculated using the Kaplan-Meier method and Greenwood formula will be used to calculate the standard errors.
- The cumulative incidence of relapse/progression and non-relapse mortality will be calculated as competing risks according to Gooley et al.[1]

Sponsor/Licensee:

City of Hope
Case Report Forms
Medidata Rave EDC®

Table of Contents

Abbreviations

Abbreviation	Meaning
AE	Adverse Event
CFR	Code of Federal Regulations
COH	City of Hope
CR	Complete Response
CRA	Clinical Research Associate
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
Cy	Cyclophosphamide
DLT	Dose Limiting Toxicity
DSMC	Data Safety Monitoring Committee
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GvHD	Graft versus Host Disease
IB	Investigator Brochure
ICF	Informed Consent Form
IDS	Investigational Drug Services
IND	Investigational New Drug
IRB	Institutional Review Board
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
PD	Progressive Disease
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease

1.0 Goals and Objectives (Scientific Aims)

In patients undergoing T-cell replete HLA-mismatched unrelated donor hematopoietic cell transplantation (HCT) for hematologic malignancy, using either an ablative or reduced intensity conditioning regimen:

1.1 Primary Objective

To estimate the GVHD-free relapse/progression-free survival (GRFS) at one-year post HCT and to evaluate the clinical activity of post-transplant high dose cyclophosphamide (PTCy).

1.2 Secondary Objectives

- To summarize toxicities/complications/infections including type, frequency, severity, attribution, time course and duration through 100 days post-transplant.
- To estimate the cumulative incidence (CI) of acute and chronic GVHD.
- To characterize the time course of neutrophil and platelet recovery/engraftment.
- To estimate overall survival (OS), progression-free survival (PFS), CI of relapse/progression and non-relapse mortality (NRM) at 100 days, 1 year and 2 years.
- To describe quality of life at 100 days, 6 months, 1 and 2 years.
- To characterize immune cell reconstitution and T cell repertoire post high dose cyclophosphamide in mismatched donor HCT.
- To characterize quality of life.

2.0 Background

2.1 Introduction/Rationale for Development

Allogeneic hematopoietic cell transplantation (HCT) is an established treatment for a large number of inherited metabolic and immune deficiencies and for benign and malignant blood and marrow conditions. However, Graft-versus-Host-Disease (GvHD) continues to be a major problem with substantial morbidity and mortality limiting the general value on the procedure. GvHD results from a complex interaction between recipient tissues and genetically disparate donor immune system.[2]

In the initial phase, damage to the host tissues results in a self-limited burst of inflammatory cytokines. Later, donor T cells recognize alloantigens presented by host antigen presenting cells (APC) leading to amplification of the systemic inflammatory response, now with contribution of donor cells. In the last phase, host tissues are subjected to damage and apoptosis driven inflammatory cytokines and cellular effectors, thus establishing a positive inflammatory feedback loop. Even in the setting of HLA-matched donors, patient and donor can differ in self-peptides derived from minor histocompatibility antigens (mHA). Those polymorphic peptides are presented by HLA class I antigens (rarely HLA class II) and can trigger the activation of donor-derived T cells. A number of these mHAs have been identified.[3] In fact, mismatches of known mHA among HLA identical donor-recipient pairs have been associated with the development of GvHD after HCT.[4]

The currently used GvHD prophylactic regimens are based on the routine use of different combinations of methotrexate, calcineurin or mTOR inhibitors, mycophenolate mofetil and

antithymocyte globulins (ATG).[5] Yet even in the setting of HLA-matched donors, clinically significant (grade II-IV) and severe (grade III-IV) acute GvHD (aGvHD) still occur in about 35-50% and 15% of cases, respectively. The latter is always associated with decreased survival.[6] Chronic GvHD (cGvHD) occurs in up to 70% of patients either after aGvHD or de novo and is also associated with substantial morbidity and mortality.[6] Risk of GvHD is further increased in those undergoing mismatched donor transplants leading to absolute decrease in survival approaching up to 10% in different studies.[6-12] Furthermore, it has been suggested that some of the drugs used in the prevention of GvHD might indeed have deleterious effects. For instance, methotrexate can independently cause tissue injury; paradoxically exacerbating the cytokine cascade associated with GvHD.[13] Also calcineurin inhibitors, by suppressing IL-2 T-cell responses can be damaging to regulatory (CD4+ CD25+ FoxP3+) T cell population. [14] The decrease in number and function of regulatory T cell is associated with worsening of cGvHD. [15,16]

Approximately 40% and up to 70% of patients with acute and chronic GvHD will have durable responses to corticosteroid therapy, respectively. Over the past 20 years, there has been little change in this response rate, despite addition or substitution of other immunosuppressive drugs to GvHD treatment regimens.[17] The prognosis of patients with steroid refractory GvHD is poor.[18] A strategy that minimizes the incidence of GvHD, without other adverse effects, would be an effective approach to improve survival after allogeneic HCT.

GvHD incidence can be decreased with various pharmacologic agents. Early transplants were done using post-transplant methotrexate to prevent GvHD; in the 1980s cyclosporine was shown to be superior to methotrexate and in 1986 the combined use of cyclosporine and methotrexate was shown to be superior to single agent prophylaxis.[19] More recently, other calcineurin-inhibitors, such as tacrolimus have been developed as GvHD prophylactic agents due to favorable toxicity profiles in comparison with cyclosporine.[20,21] In fact, phase III trials comparing tacrolimus/methotrexate versus cyclosporine/methotrexate for related and unrelated donors have been performed. In the unrelated donor setting, the incidence of grade II-IV acute GvHD was 56% among the 46 patients randomized to tacrolimus arm versus 74% among the 63 patients randomized to cyclosporine arm.[22] Currently, the combination of tacrolimus/methotrexate remains a standard for GvHD prophylaxis, despite its limited efficacy. However, improved GvHD prophylaxis is a significant clinical need in HCT.

2.1.1 Rationale for mismatched unrelated donor (MMUD) and suboptimal outcome

Only 30% of patients in need of HCT have an available matched sibling donor (MSD), therefore the majority of patients who are in need of this procedure will have to undergo an alternative donor search. Despite the increasing number of unrelated volunteer donors (UD) available through the National Marrow Donor Program (NMDP), significant number of patients will not have a well-matched (at least; 8/8 HLA-A, B-, C-, DRB1) unrelated donor (MUD).[23] The likelihood of finding a matched donor through the registry varies among racial and ethnic groups, with the highest probability among whites of European descent (at 75%), and the lowest probability among blacks of south or Central American descent (at 16%).[23] For those who will not have a matched donor available to them, options include mismatched unrelated (MMUD), cord blood, or haploidentical donors. Early studies of HCT with alterative donor sources have reported worse outcome due to poor engraftment, delayed immune reconstitution with increased risk of infection, and high incidence of GvHD. Progress in the last decade has significantly improved the outcome of alternative donor HCT. This progress is attributed to better donor selection, vigorous and on-target GvHD prophylaxis, and the introduction of more suitable

preparative regimens. These advances make the alternative donor HCT a feasible option for patients who are in need of procedure and lack a suitable matched donor.

In previously reported registry studies, MMUD HCT has been associated with inferior transplant outcomes regardless of preparative regimen intensity,[8,24,25] and graft source [bone marrow (BM) or peripheral blood stem cell (PBSC)][8,10,26] used. This was largely due to increased risk of GvHD and NRM resulting in inferior OS. This deleterious effect was seen across all disease categories, except possibly the high-risk group where risk of disease relapse significantly outweighs risk of TRM.[24] This was true with most standard GvHD prophylaxis regimens of methotrexate combined with a calcineurin inhibitor (CI). When the novel tacrolimus/sirolimus (T/S)-based GvHD prophylaxis regimen was compared with "standard of care" methotrexate/tacrolimus (M/T) in a phase III randomized study, results were equivalent in the setting of fully matched related donor alloHCT.[27] Smaller single center phase II randomized study including both MSD and MUD showed similar outcomes with the advantage of decreasing grade II-IV aGvHD and possibly moderate to severe chronic GvHD.[28]

With promising results in matched donor HCT, it has been used in the setting of MMUD with or without additional agents (ATG, Methotrexate, bortezomib, etc.). The outcome of this group of patients was never studied systemically until recently when it was presented in large retrospective cohort by our group (Al Malki, MM. et al. BBMT 2016; Abstract 7712). In this study, we evaluated a consecutive case-series of 482 patients who underwent URD HCT at City of Hope from 2005 to 2013 using a MUD (8/8 matched at HLA-A, B, C, and DR: n=351) or MMUD (≤7/8 match: n=131). Mini-MTX was added in 25% of patients, ATG in 6.4%, or both in 1.8%. Conditioning was myeloablative in 45% of patients. Indication for HCT was acute leukemia (n=308), MDS/MPN (n=69), NHL (n=68), HL (n=4), CLL (n=12), CML (n=16), and multiple myeloma (n=5). Disease risk was low in 38%, intermediate in 22% and high in 40% of the patients. With the exception of age (median 54.6 [range: 18.0-73.8] for MUD, 45.2 [range: 18.7-71.8] for MMUD, p<0.01), conditioning intensity (MAC: 41.0% in MUD, 55.7% in MMUD, p<0.01), and the use of additional agent for GVHD prophylaxis (21% in MUD, 69% in MMUD, p<0.01) pre-HCT patient/disease characteristics were similar. With the median follow-up of 3.3 years (range: 0.6-8.9) for surviving patients, OS was significantly lower in the MMUD group (at 3-years: 46% [95%CI: 36-54] and 59% [95%CI: 54-64] respectively, p=0.009, **Figure 1**)

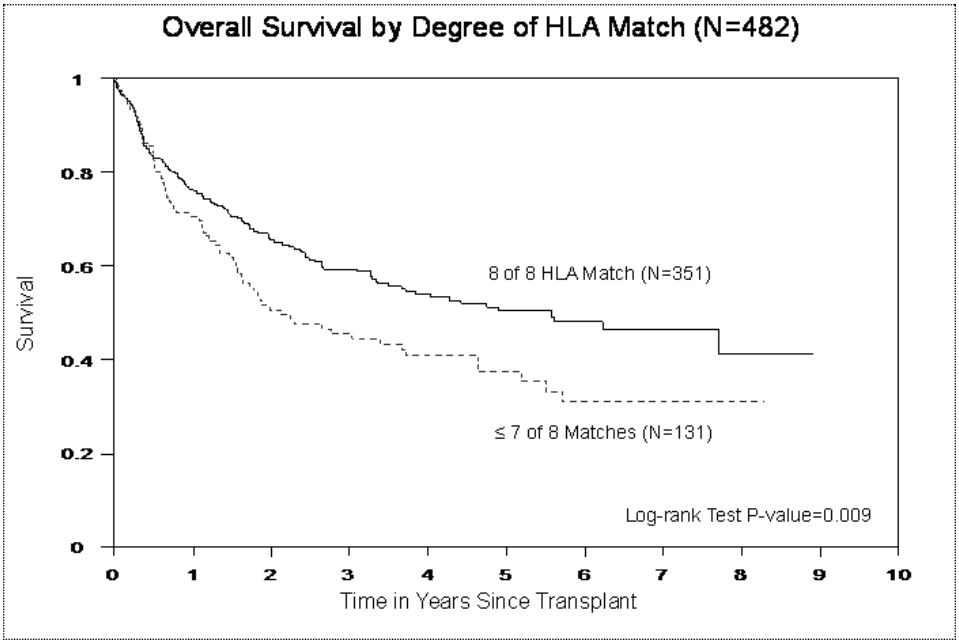


Figure 1. Overall Survival post-transplant based on HLA matching

primarily due to increased non-relapse mortality (34% vs. 19% p=0.002). While differences were not seen in relapse, aGvHD (grade 2-4 or 3-4) or cGvHD (limited or extensive), death caused by cGvHD and infection was significantly greater in MMUD (13% vs. 7%, p=0.02, 11% vs. 5%, p<0.01; respectively). By multivariable analysis, MMUD (HR=1.4 [95%CI: 1.1-1.9] p=0.02) and high-risk disease (HR= 1.9 [CI: 1.4-2.5] p<0.01) were predictive factors for OS.

Therefore, novel GvHD prophylaxis regimens are needed to overcome the inferior outcome in this group of patients who receive MMUD HCT.

2.1.2 Rationale for Post-transplant Cyclophosphamide for GvHD prophylaxis

High dose cyclophosphamide is a potent immunosuppressive agent that has been successfully used to prevent GvHD in unrelated, HLA-matched sibling and haploidentical bone marrow/PBSC transplants in single center as well as in multi-center studies.[29-34] Preclinical studies have shown that cyclophosphamide administered early post HCT preferentially kills activated alloreactive T cells while sparing resting, non-alloreactive T cells leading to suppression of GvHD as well as increased rates of graft rejection.[30] Furthermore, a recent study showed that human regulatory T cells are resistant to PTCy and contribute to its GvHD preventive effects.[35]

Recently, cyclophosphamide has been administered in high doses after transplantation as the only GvHD prevention strategy both in the setting of myeloablative and reduced intensity conditioning.[29] Cyclophosphamide inhibits rapidly proliferating T cells in a manner similar to methotrexate.[36] However, the agent spares the hematopoietic stem cells and gastro- intestinal tract because of their high content in aldehyde dehydrogenase (AD), which converts 4-hydroxcyclophosphamide into a non-alkylating metabolite.[37] In a mouse model, the drug fostered conversion of naïve CD4 + T cells into regulatory T cells (Ganguly, S. et al. Blood 2010; Abstract 3749).

2.1.2.1 Post-transplant Cyclophosphamide in Haploidentical Donor Transplants

Based on promising pre-clinical results at Johns Hopkins, a Phase I/II clinical trial of haploidentical HCT to treat high-risk hematologic malignancies was initiated in 1999.[38,39] Following a non-myeloablative (NMA) regimen of fludarabine, cyclophosphamide, and low-dose TBI, GvHD prophylaxis consisted of cyclophosphamide (PTCy) given on Days +3 and +4 post-transplant, tacrolimus, and mycophenolate mofetil (MMF) (**Figure 2**).[33] Primary graft failure

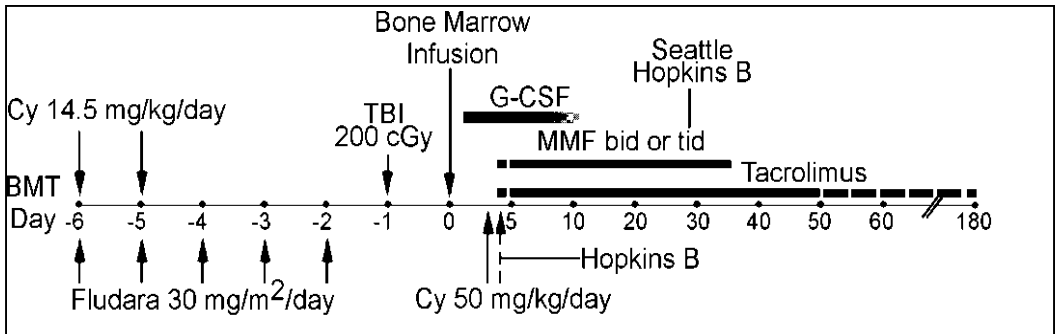


Figure 2: Treatment schema of patients undergoing haploidentical donor transplants in the BMT CTN 0603 clinical trial.

occurred in 13% of patients, and was fatal due to infection in one patient in whom autologous hematopoiesis failed to occur. In general, complete T-cell engraftment was observed by Day +28 or the grafts were rejected. Cumulative incidences of grades II-IV and grades III-IV acute GvHD by Day 200 were 34% and 6%, respectively. There was lower incidence of extensive chronic GVHD among recipients of two versus one dose of PTCy (5% versus 25%; p=.05). There was no difference in the incidence of severe acute GvHD with one or two doses of PTCy. The cumulative incidences of non-relapse mortality and relapse at 1 year were 15% and 51%, respectively. Overall and event-free survivals (EFS) at two years after transplantation were 36% and 26%, respectively. Patients with lymphoid malignancies appeared to have improved EFS compared to those with myeloid malignancies (p=0.02).

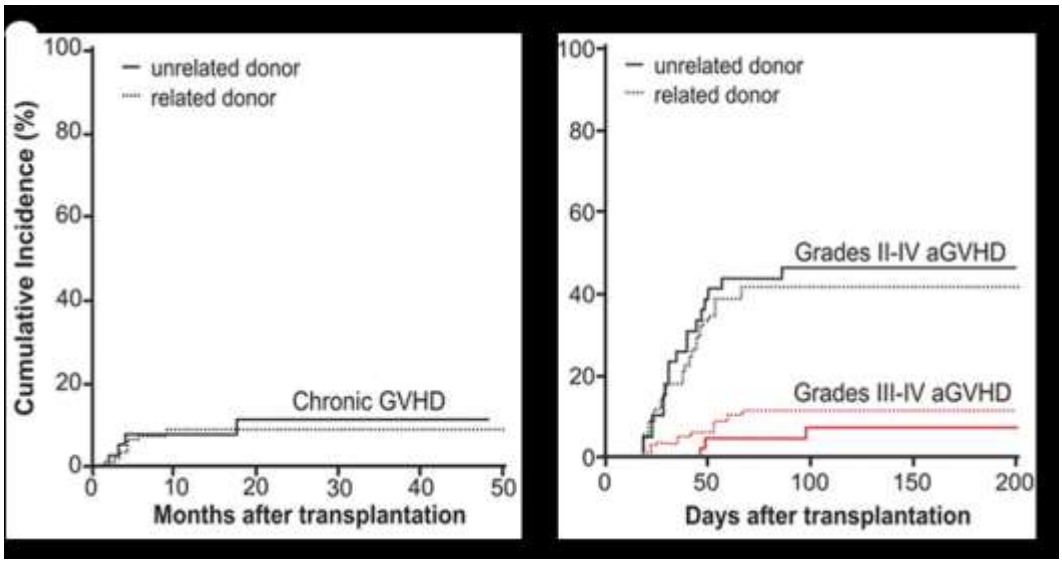
The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) sponsored a multi-center Phase II trial of haploidentical BMT (BMT CTN 0603) for high-risk hematologic malignancies modeled after the Hopkins approach. This was published along with a similar study using cord blood grafts without PTCy (BMT CTN 0604).[32] The 1-year probabilities of OS and PFS were 54% and 46% after cord transplantation and 62% and 48% after haploidentical bone marrow transplantation.

The Day +56 cumulative incidence of neutrophil recovery was 94% after double umbilical cord blood and 96% after haploidentical marrow transplants. The 100-day cumulative incidence of grade II-IV acute GVHD was 40% with cord blood and 32% with haploidentical bone marrow. The 1-year cumulative incidences of NRM and relapse after cord transplantation were 24% and 31%, respectively; corresponding rates after haploidentical bone marrow transplantation were 7% and 45%.

The use of PBSC instead of marrow may allow wider applicability of this approach but there is concern about higher risks of acute and chronic GVHD due to the 5-10-fold higher number of T-cells in the allograft. Recently, groups from Atlanta, Houston and London reported studies in which PBSC were substituted for bone marrow with PTCy in the haploidentical donor setting. **Error! Bookmark not defined.**[40-42] In those studies, the incidences of severe acute GVHD, chronic GVHD and non-relapse mortality at 1 year with PBSC were comparable to the rates seen with bone marrow.

2.1.2.2 PTCy in HLA Matched Donors

After the success of PTCy in the prevention of GvHD in the setting of haploidentical transplantation, Luznik et al[29] reported a large study of patients who underwent allogeneic HCT from HLA-matched donors after MA conditioning using busulfan-cyclophosphamide (BuCy) and PTCy as a single agent for prophylaxis of GvHD. A total of 117 patients with high-risk hematologic malignancies were transplanted from MSD (n=78) or MUD (n=39). Half of the patients were not in remission at the time of transplant. Bone marrow grafts were used for all patients. The incidence of GvHD was remarkably low (43% grades II-IV acute, 10% grades III/IV acute, 10% chronic) showing the effectiveness of the approach (**Figure 3**). There was no difference in the incidence of acute or chronic GvHD between MSD and MUD. Almost two-thirds of the patients did not require any additional immunosuppressive therapy after PTCy. Patients who did develop GvHD responded to steroids alone in 20% of cases or steroids plus a second agent (calcineurin or non-calcineurin) in 75% of cases. Rates of grade II-IV acute and chronic GVHD were both 10%. Observed rates of non-relapse mortality and disease relapse were 20% and 44%, respectively. This study showed that PTCy was effective as a single-agent to prevent severe acute or chronic GvHD in the vast majority of patients undergoing MAC HCT from matched donors. Only 3% of deaths were due to infection suggesting that immune



reconstitution is robust in such patients. Obviating the need for ongoing immunosuppression post-transplant provides an optimal platform for cellular therapy to prevent or treat relapsed disease.

Figure 3. GVHD incidence after a myeloablative post-transplant Cy regimen (Luznik et al.[29])

These encouraging outcomes with PTCy in prevention of GvHD were recently reproduced in a multi-institutional study that effectively combined this novel single-agent short-course GvHD prevention strategy in combination with IV Bu/Flu MA conditioning. In this study,[43] 92 adult patients (median age 49; range=21-65) with high-risk hematologic malignancies were enrolled at three centers. Forty-five (49%) patients received related allografts, and 47 (51%) received unrelated allografts. GvHD prophylaxis was solely with PTCy at 50 mg/kg/day on post-transplant days +3 and +4 after bone marrow allografting. The cumulative incidences of grade II-IV acute, grade III-IV acute, and chronic GVHD were 51%, 15%, and 14%, respectively. NRM at 100 days and 1 year were 9% and 16%, respectively. With a 2.2 year median follow-up, the two-year EFS and OS were 62% and 67%, respectively. Donor type did not impact on NRM, EFS, or OS. Patients in complete remission (CR) without evidence of minimal residual disease had remarkably high rates of EFS (80%) and OS (79%).

Champlin, R. el al reported a similar GvHD preventive strategy in the setting of RIC combining fludarabine, busulfan with or without rabbit ATG in a group of 31 older and medically frail patients receiving matched related or unrelated donor transplant (Alousi, A. et al. Blood 2010; Abstract 2314). The incidence of grade III and IV acute GvHD was 13% and of chronic GvHD 11%. Primary and secondary graft failure occurred in 1 and 2 patients respectively. The 1-year OS of was impressive for such a high-risk population.

In another study conducted by the group in Atlanta[44], PTCy was combined with a brief course of sirolimus in a calcineurin inhibitor-free regimen. Twenty-six patients (median age, 61 years) underwent unmanipulated PBSC HCT from an 8/8 locus-matched donor (MRD= 17; MUD= 9). Donor engraftment occurred in all patients. The cumulative incidence of grade II-IV acute GVHD, grade III-IV acute GVHD, and chronic GVHD was 46%, 15%, and 31% respectively. One-year NRM was 4%. The median time to immunosuppression discontinuation was day +138. With a median follow-up of 20 months, the estimated 2-year overall survival was 71%, estimated disease-free survival was 64%, and estimated relapse incidence was 32%. In patients

with a lymphoid malignancy (e.g., chronic lymphoblastic leukemia, non-Hodgkin lymphoma, Hodgkin disease), 2-year disease-free survival was 100%, and there were no relapses. Good immune reconstitution was evidenced by low cytomegalovirus reactivation rate of 21% (n=4/19).

Al-Homsi, A. et al. reported feasibility of phase I combining PTCy with a brief course of proteasome inhibitor (i.e. bortezomib)[45], in this study 15 patients underwent reduced-intensity PBSC HCT from MSD or MUD. Cyclophosphamide was given at a fixed dose (50 mg/kg on days +3 and +4). Bortezomib dose was started at 0.7 mg/m^2, escalated up to 1.3 mg/m2, and was administered on days 0 and +3. Patients receiving grafts from MUD also received rabbit ATG. The combination was well tolerated and allowed prompt engraftment in all patients. The incidences of acute GVHD grades II to IV and grades III and IV were 20% and 6.7%, respectively. With a median follow-up of 9.1 months (range, 4.3 to 26.7), treatment-related mortality was 13.5% with predicted 2-year disease-free survival and overall survival of 55.7% and 68%, respectively.

With the excellent results obtained in these studies and the ineffectiveness of the available "standard" GvHD prophylaxis regimens in overcoming the deleterious effect of HLA-mismatched donors, we expect that the use of PTCy for GvHD prophylaxis as a novel regimen will improve outcomes of patients undergoing MMUD HCT in this clinical trial.

2.1.3 Immune reconstitution after PTCy

The ability to shorten the duration of post-grafting immunosuppression after HLA-matched allogeneic HCT with PTCy was marked by prompt immune reconstitution and a low incidence of opportunistic infections. Among 47 patients studied immune-phenotypically after HLA-matched HCT; the mean CD4$^+$ T cell count on day 30 was 98 cells/µl and on day 60 was 124 cells/µl.[35] At both days 30 and 60 after HCT, the CD8$^+$ T cell counts were already within the normal range. Effector regulatory T cells rapidly recovered to donor levels by 30 days after HCT, and there was favorable memory CD4$^+$ T cell recovery compared with naïve CD4$^+$ T cells.

In the above-described studies,[29,43] no patients died of CMV or invasive fungal infection. Reactivation of CMV occurred in 29% of patients, and there were only two documented cases of CMV disease. The rapid recovery of CMV-specific immunity correlated with the results of *in vitro* ELISPOT assays. The frequency of cells secreting interferon gamma in response to stimulation with pentadeca-peptides of the immune-dominant CMV protein, pp65, at day 30–60 after alloHCT did not differ from pre-transplantation specimens from CMV-seropositive donor/recipient pairs. The absence of post-transplantation Epstein–Barr virus-associated lymphoproliferative disease is another indicator of the prompt immunologic recovery seen with PTCy. [46]

Immune reconstitution after PTCy was studied in the setting of Haploidentical HCT and has been shown to be comparable to immune reconstitution in matched donor (MSD and MUD) undergoing transplant with "standard" GvHD prophylaxis regimen.[35,46,47] But this needs to be studied in the setting of MMUD, which is going to be one of the objectives of this clinical trial as well.

2.2 Overview of Proposed Study

This is a single center, pilot/estimation study to evaluate the clinical activity associated with the use of PTCy in preventing severe grades of acute/chronic GvHD and reducing NRM/PFS in patients with hematological disease receiving an HLA-mismatched unrelated donor HCT after either myeloablative or reduced-intensity conditioning regimen. The overarching goal is to

assess the clinical activity associated with the addition of PTCy through estimation of GRFS at 1 year overall and by conditioning regimen (MAC and RIC).

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

3.0 Patient Eligibility

3.1 Patient Inclusion Criteria

1. Age Criteria: 5 to 75 years of age if meets criteria for HCT per SOP
2. Patients with acute leukemia or chronic myelogenous leukemia with no circulating blasts and with less than 10% blasts in the bone marrow.
3. Patients with myelodysplastic syndrome [MDS] with intermediate-2 or high risk per IPSS (or intermediate, high, very high risk by IPSS-R) or myeloproliferative neoplasm; primary or secondary if high-risk features or refractory disease.
4. Patients with chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular, marginal zone, diffuse large B-cell, Hodgkin Lymphoma, or mantle cell lymphoma with chemosensitive disease at time of transplantation. All types of lymphoma are eligible.
5. High risk, or refractory and relapsed Multiple Myeloma.
6. No available suitable HLA-matched related donor
7. Available matched unrelated donor (see Section 3.2)
8. Cardiac function: Ejection fraction at rest ≥ 50%.
9. Karnofsky Performance Status (KPS) ≥70 (Appendix D)
10. Measured creatinine clearance more than 60 mL/min. The updated Schwartz formula should be used for pediatric patients (≥5 to 12 years old.
11. Pulmonary function: DLCO ≥ 50% (adjusted for hemoglobin) and FEV1 ≥ 50%.
12. Liver function: total bilirubin < 1.5 x the upper limit of normal and ALT/AST and alkaline phosphatase < 2.5x the upper normal limit. Patients who have been diagnosed with Gilbert's Disease are allowed to exceed the defined bilirubin value of 1.5x the upper limit of normal.
13. Female subjects (unless postmenopausal for at least 1 year before the screening visit, or surgically sterilized), agree to practice two (2) effective methods of contraception at the same time, or agree to completely abstain from heterosexual intercourse, from the time of signing the informed consent through 12 months post-transplant.
14. Male subjects (even if surgically sterilized), of partners of women of childbearing potential must agree to one of the following: practice effective barrier contraception, or abstain from heterosexual intercourse from the time of signing the informed consent through 12 months post-transplant.
15. All subjects must have the ability to understand and the willingness to sign a written informed consent.

3.2 Donor Inclusion Criteria

1. 7 out of 8 at high resolution using DNA-based typing with either antigen or allele mismatched HLA (-A, -B, -C, and -DR) or 8/8 HLA-matched with either double DQ mismatch (10/12) or combined DQ and DP mismatch.
2. Donor must be willing to donate peripheral blood stem cells.
3. Suitable Donor – Medically cleared to donate per NMDP.
4. Absence of DSA to the mismatched HLA- locus.
5. Donor choices per MUD committee according to center SOP.

3.3 Patient Exclusion Criteria

1. Prior allogeneic transplant.
2. Active CNS involvement by malignant cells.
3. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression or no clinical improvement) at time of enrollment.
4. Patients with transformed lymphoma (e.g., Richter's transformation arising in follicular lymphoma or chronic lymphocytic leukemia).
5. Patients seropositive for the human immunodeficiency virus (HIV).
6. Patient with active Hepatitis B or C determined by polymerase chain reaction (PCR).
7. Myocardial infarction within 6 months prior to enrollment or New York Heart Association (NYHA) Class III or IV heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at screening must be documented by the investigator as not medically relevant.
8. Female patients who are lactating or pregnant.
9. Patients with a serious medical or psychiatric illness likely to interfere with participation in this clinical study
10. History of another primary malignancy that has not been in remission for at least 3 years (the following are exempt from the 3-year limit: non-melanoma skin cancer, fully excised melanoma in situ [Stage 0], curatively treated localized prostate cancer, and cervical or breast carcinoma in situ on biopsy or a squamous intraepithelial lesion on PAP smear).
11. Psychosocial issues: no appropriate caregivers identified, or non-compliant to medications
12. Subjects, who in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.

3.4 Inclusion of Women and Minorities

The study is open anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue approximately 38 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

3.5 **Co-enrollment with other HCT trials**

In principle co-enrollment with other interventional trials is allowed including therapeutic trials for GVHD.

4.0 Screening and Registration Procedures

4.1 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Reference is made to Section 10.0 – Study Calendar.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record.

4.3 Registration Requirements/Process

To register a subject, the subsequent procedure is to be followed.

1. The data manager/coordinator/research nurse should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window.
2. The data manager/coordinator/research nurse should then e-mail copies to DCC@coh.org of the following documents to the DCC:
    - Completed Eligibility Criteria List
    - Source documentation to support eligibility criteria**
    - Signed informed consent document
    - Signed HIPAA authorization form (if separate from the informed consent document)
    - Signed subject's Bill of Rights

** Provide copies of source documentation only if not readily available as a finalized record in the COH Electronic Medical Record (EMR).

3. After having received all transferred documentation, the DCC will complete the review the documents to verify eligibility, working with the CRC/Protocol Nurse as needed to resolve any missing required source elements. A participant failing to meet all protocol eligibility requirements will not be registered.
4. Once eligibility has been confirmed, DCC staff will register the participant by: assigning a subject accession number, register the subject on study centrally into

COH clinical trials management system and enter the subject into the eCRF system, Medidata RAVE.

5. Once registration has been completed, DCC staff will send a Confirmation of Registration Form, including the participant study number to the study team.

4.4 Screen Failures and Registered Participants Who Do Not begin Study Treatment

The DCC is to be notified of all participants who sign consent but do not meet eligibility criteria or do not initiate study treatment.

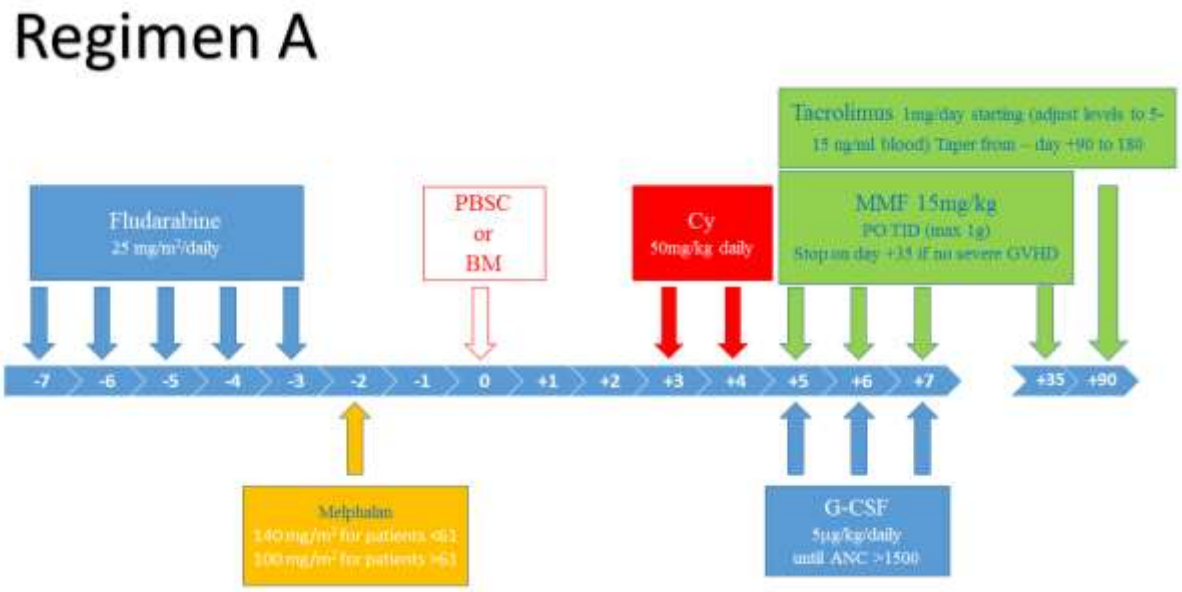
4.5 Randomization and/or Dose Level Assignment

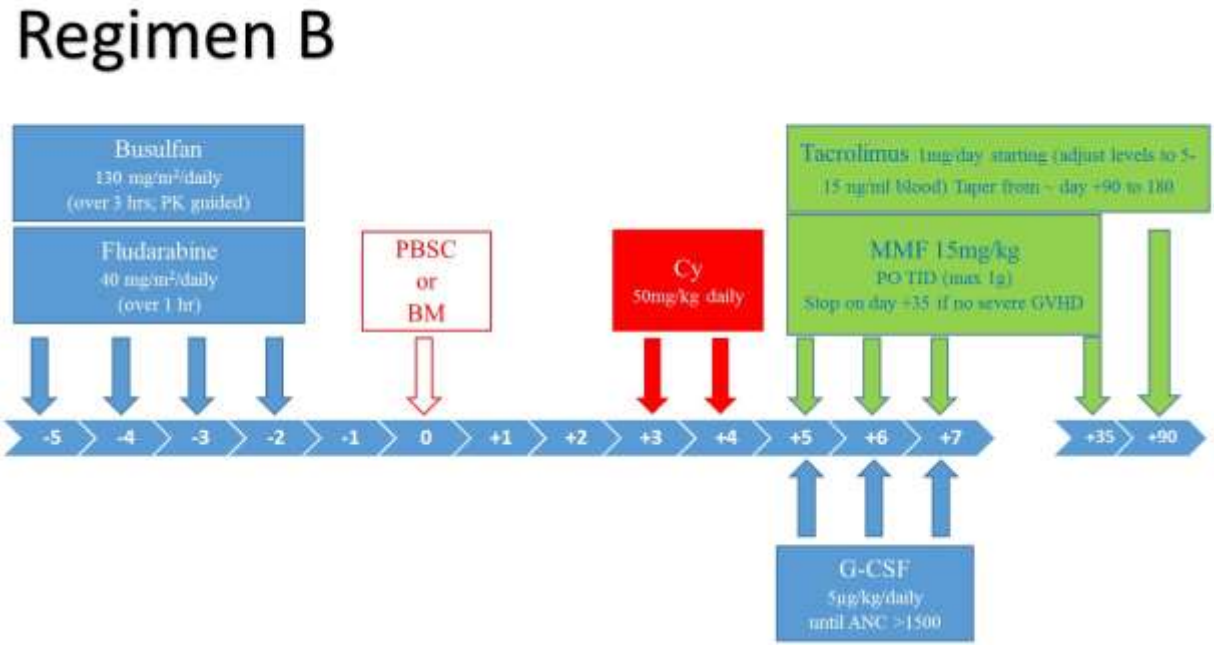
Not applicable

5.0 Treatment Program

5.1 Study Schema

Note: A window of 1-2 days is allowed for stem cell availability.





5.2 Treatment Overview

Note: a window of 1-2 days is allowed for stem cell availability. A window of ±7 days is allowed for outpatient procedures before day +100. For outpatient procedures between day +100 and +270, a window of 14 days; and for outpatient procedures after day +365, a window of 30 days is allowed.

5.2.1 Conditioning Regimens

Eligible patients will receive either myeloablative (MA) or reduced intensity (RI) conditioning regimen following guidelines per institutional SOP and according to the discretion of the attending physician and principle investigator as shown in the Table below. Other regimens not included in the Table, might be considered after review by the principle investigator. Patients are typically admitted to the hospital the day before the conditioning regimen begins and remain inpatient until after engraftment of neutrophils is verified (~2-3 weeks) and they are able to eat. After discharge from the hospital, remaining visits and tests are outpatient unless problems are encountered.

TABLE: CONDITIONING REGIMENS

Reduced Intensity (RIC) Conditioning	**Myeloablative (MAC) Conditioning**
Fludarabine/Melphalan (Flu/Mel) • Fludarabine 25 mg/m^2 IV Days –7, –6, –5, –4, –3 • Melphalan 140 mg/m2 IV Day -2 (or 100 mg/m^2 IV if age ≥60)	**Fludarabine /Total Body Irradiation (Flu/TBI)** • Fludarabine 30 mg/m^2 IV Days -7,-6,-5 • FTBI 150 cGy twice a day Days -4, -3, -2, -1 (total 1200 cGy)
	Fludarabine/ Busulfan (Flu/Bu)

	• Fludarabine 40 mg/m^2 IV Days -5, -4, -3, -2 • Busulfan 130 mg/m^2 Days -5, -4, -3, -2 (PK-guided)

5.2.1.1 Fludarabine/Melphalan (Flu/Mel)

The allowable Flu/Mel regimen is the following:

- Fludarabine 25 mg/m^2/day infused IV over 60 minutes on Days –7, –6, –5, –4, –3
- Melphalan 140 mg/m^2 IV (or 100 mg/m^2 IV if age ≥60) Day -2

The sequence of fludarabine and melphalan administration will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above.

5.2.1.2 Fludarabine /Total Body Irradiation (Flu/FTBI)

The allowable FluFTBI regimen is the following:

- Fludarabine 30 mg/m^2 IV infused IV over 60 minutes on Days -7, -6, -5
- FTBI is administered at a dose rate of < 20 cGy/minute. Doses of 150 cGy/ fraction are administered at a minimum interval of 4 hours between fractions, twice a day for a total of 8 doses (1200 cGy) over 4 days (Day -4, -3, -2 and -1). Sequential doses are administered in an anterior/posterior or lateral orientation. The orientation of FTBI chosen will be left to the discretion of the radiation oncology specialist. In addition, for male patients receiving transplants for ALL or AML, the use of boost to the testes is allowed according to institutional practices. General anesthesia is allowed (e.g., young children).

5.2.1.3 Fludarabine/ Busulfan (Flu/Bu)

The allowable FluBu regimen is the following:

- Days -5 to -2: 130 mg/m^2 this will be infused over 3 hrs. Daily for 4 days; total dose of 520 mg/m^2. Busulfan should be administered per institutional guidelines and PK guidance is advisable.
- Days -5 to -2: Flu 40 mg/m^2 this will be infused over 1 hrs. Daily for 4 days, total dose of 160 mg/m^2.

The sequence of busulfan and fludarabine administration will be done according to institutional standards as long as the prescribed doses are the same as the allowable regimen above. **Only IV Busulfan is allowed.** For patients receiving busulfan doses according to pharmacokinetics, targeting doses to area under the curve of 4000 µMol/min or less is allowed.

For CNS seizure prophylaxis, the use of Keppra is allowed. Phenytoin and other potent CYP3A4 inducers are not allowed for seizure prophylaxis to avoid drug interactions.

5.2.2 Hematopoietic Cell Graft Source

Mobilized PBSC is the preferred graft source for patients enrolled in this clinical trial. BM could be used alternatively at the discretion of the attending physician and principle investigator

- Donors will undergo G-CSF mobilization according to institutional and donor center practices. PBSC will be collected by apheresis according to local institutional guidelines. Plasma and red cell depletion are allowed for volume reduction or ABO incompatibility but any other form of graft manipulation (including ex-vivo T cell depletion) is not permitted. The target stem cell dose is between 2 x 10^6/kg and 6 x 10^6/kg (actual body weight) $CD34^+$ cells. The maximum CD34+ cell dose is 6 x 10^6/kg. Up to two leukapheresis procedures may be performed to obtain the minimum $CD34^+$ cell target. If, after two leukapheresis procedures, fewer than 2 x 10^6/kg $CD34^+$ cells have been collected, transplant centers will have the discretion to continue PBSC cell harvesting or to proceed to bone marrow harvesting to obtain sufficient cells. If more than 6 x 10^6/kg $CD34^+$ stem cells are collected, the excess will be cryopreserved for future use, but will not be administered to the patient.

  PBSC will be administered on Day 0 to all patients according to institutional guidelines after appropriate processing and quantification has been performed by the local laboratory. Stem cells are administered through an indwelling central venous catheter. If infusion occurs over two days, Day 0 is the day the last infusion is completed.

- Bone marrow harvest can be done under either general or regional (epidural, spinal) anesthesia. Donor bone marrow will be harvested with a target yield of 4 x 10^8 TNC/kg recipient ideal body weight (IBW). The bone marrow graft will not be manipulated to deplete T cells. Patients will receive unprocessed marrow unless there is an ABO incompatibility, in which case red blood cells or plasma will be depleted from the donor marrow using institutional practices. Processing for reduction of volume, plasma or fat may be performed by the transplant center according to institutional guidelines.

5.2.3 GvHD prophylaxis (Investigational part)

5.2.3.1 *Tacrolimus/Mycophenolate Mofetil/Cyclophosphamide*

5.2.3.1.1 Tacrolimus

Tacrolimus will be given by continuous intravenous infusion at flat dose of 1 mg/day starting Day +5. Serum levels of tacrolimus will be measured at Day 7 and then should be checked weekly at least thereafter, and the dose adjusted accordingly to maintain a suggested level of 5-15 ng/mL. Tacrolimus taper can be initiated at a minimum of 90 days post HCT if there is no evidence of active GVHD. The rate of tapering will be done according to institutional practices but patients should be off tacrolimus by Day 180 post HSCT if there is no evidence of active GVHD.

Dose reductions should be made if toxicity is present or whole blood levels are above the recommended range, in the absence of toxicity. Patients with severe intolerance of tacrolimus may be placed on cyclosporine (trough level of 200-400 ng/mL) or sirolimus (trough level of 3-8 ng/mL).

5.2.3.1.2 Mycophenolate mofetil (MMF)

MMF will be given at a dose of 15 mg/kg TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 grams (1g TID, IV or PO). MMF prophylaxis will start Day 5 and discontinue after the last dose on Day 35, or may be continued if active GVHD is present.

5.2.3.1.3 Cyclophosphamide

Hydration and Mesna support prior to and after cyclophosphamide may be given according to institutional standards. Cyclophosphamide will be given on **Day 3 post-transplant** (between 68 and 72 hours after the start of the HPC infusion) and on Day 4 post-transplant (24 hours after Day 3 cyclophosphamide). Cyclophosphamide will be given as an IV infusion over 1-2 hours (depending on volume) at a dose of 50 mg/kg ideal body weight (BW); if Actual BW < Ideal BW, use Actual BW. If Actual BW>125% of IBW, use Adjusted BW].

It is crucial that no immunosuppressive agents are given prior to transplant, or until 24 hours after the completion of the post-transplant cyclophosphamide. This includes corticosteroids as anti-emetics.

5.2.3.2 G-CSF Support

G-CSF 5 µg/kg/day will be given beginning Day +5 until ANC >1,500/mm^3 for 3 consecutive days. The G-CSF dose may be rounded per institutional guidelines.

5.3 Planned Duration of Therapy

Initial therapy (transplant conditioning, stem cell infusion, start of GVHD prophylaxis) will require 3-4 weeks of inpatient treatment).

5.4 Criteria for Removal from Treatment

Disease relapse or progression and non-compliance with protocol are grounds for removal from treatment.

5.5 Subject Follow-Up

Outpatient follow-up will be twice weekly for the first 100 days post-transplant, twice monthly until 6 months post-transplant, and monthly until the patient is off immunosuppressive therapy without evidence of GVHD (see Study Calendar Section 10.0), with at least yearly study follow-up extending 2 years beyond the date of stem cell infusion. Patients will be co-enrolled in the COH long-term follow-up protocol for allogeneic transplantation (Protocol #00029). For relapse patients, follow-up at each time point will be for survival only.

5.5.1 Quality of life (QOL) assessment

QOL assessments include self-reported patient questionnaires: SF-36, FACT-BMT, and MDASI for English and Spanish speaking patients > 18 years, and PedsQL Stem Cell Transplant Module for English speaking pediatric patients (ages 8 through 18 years). Patients will be asked to fill out QOL assessment forms at 100 days, 6 months, and 18 months and 2 years post-HCT.

5.6 Supportive Care, Other Concomitant Therapy, Prohibited Medications

5.6.1 Supportive Care

Dietary, anti-infective prophylaxis and blood product support will comply with COH **HCT** SOPs.

5.6.2 Concomitant Therapy/Prohibited Medications

It is crucial that no immunosuppressive agents are given prior to transplant, or until 24 hours after the completion of the post-transplant cyclophosphamide. This includes corticosteroids as anti-emetics.

5.7 Additional Studies

We will study the reconstitution of the immune system after T-replete HCT with PTCy as well as rate and severity of infectious disease complications.

5.7.1 Laboratory Studies

See Section 10.0 – Study Calendar for a complete list of required laboratory procedures. Correlative studies including immune cell reconstitution studies (T and B cells, and NK cells)-TCR repertoire, and assessment of plasma cytokines and GVHD biomarkers (described in Section 9.0) will be performed on patient peripheral blood and plasma samples at pre-transplant, and post-transplant on days7, 14, 21, 28 (±2 days), 42, 63 (± 7 days) 100 and 180 (± 14 days), and, 1 and 2 years (± 30 days) post-transplant.

6.0 Expected Toxicities, Dose Delays/Modifications for Adverse Events

6.1 Expected Toxicities (most severe and/or persistent)

HCT conditioning consists of high dose combination chemotherapy and may results in multiple grade 3 and 4 toxicities of both hematological and non-hematological systems by CTCAE v4.03. Transplant toxicities are captured using the Modified Bearman Scale for transplant toxicities[48]. See Section 8 for toxicities specific to the agents used for conditioning. Generalized potentially lethal or irreversible toxicities expected in allogeneic HCT are listed here.

6.1.1 Myelosuppression

All permitted HCT conditioning regimens cause potentially lethal myelosuppression that requires stem cell infusion for replacement of blood and immune functions. If the infused stem cells fail to engraft, death may result.

6.1.2 Graft Infusion Reactions

Symptoms may include changes in heart rate and/or rhythm, changes in blood pressure, fever, chills, sweats, nausea, vomiting, diarrhea, abdominal cramping, hemoglobinuria, acute renal failure, allergic reactions, respiratory dysfunction, or headache.

6.1.3 Infections

Transplantation puts the patient at higher risk for bacterial, viral, or fungal infections, which are potentially life-threatening. Prophylaxis will be initiated and patients will be closely monitored for signs of infections and will receive early and appropriate treatment.

6.1.4 Graft-versus-host Disease

After allogeneic transplantation, chronic GVHD may develop that can be disabling and can lead to death. GVHD is thought to be initiated by T-cells contained in the graft. PTCy reduce the number of alloreactive T-cells but GVHD can still occur after transplant.

6.1.5 Sinusoidal Obstruction Syndrome (SOS)/ Veno-occlusive Disease (VOD) of the Liver

SOS/VOD is a manifestation of damage to the liver by the conditioning regimen that usually develops within two weeks after allogeneic transplant and is characterized by at least two of the following:

- Hyperbilirubinemia (total bilirubin > 2 mg/dL)
- Hepatomegaly or right upper quadrant pain, or
- Sudden weight gain (> 5% above baseline)

Recipients developing SOS/VOD will receive appropriate supportive care and careful fluid management.

6.1.6 End Organ Damage

End organ damage of any or all of the major organs, including the brain, may occur as a result of cumulative toxicity from anti-neoplastic therapy, reactions to other drugs, and as a result of destructive processes (e.g., infection, GVHD, etc.) and may have a fatal outcome. Toxicities may occur in any individual patient due to multiple events and cumulative effects that may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function

6.1.7 Lymphoproliferative Syndrome

Recipients of allogeneic grafts have an increased risk of developing post-transplant lymphoproliferative disorder (PTLD) caused by EBV especially in the case of long-term immunosuppression therapy. Patients who develop a fever of unknown origin to > 39°C, lymphadenopathy, or hepatosplenomegaly, should undergo CT scanning of the chest and abdomen and/or PET scan to rule out or stage EBV PTLD. Tissue diagnosis that includes EBER and LMP-1 IHC should be attempted. Other diagnostic or staging studies will be performed as clinically indicated. EBV PTLD may rapidly progress and can be fatal if not treated. Management of suspected EBV PTLD should be discussed with principal investigator of the study. EBV PTLD can be treated with rituximab with or without chemotherapy. It is recommended that patients with increased EBV DNA levels receive rituximab pre-emptively.

6.1.8 Death

There is an approximate 5-10% risk of transplant-related mortality within the first month of transplant due to the risk of severe regimen-related toxicity, hemorrhage, opportunistic infection, or other complications. It is not expected that the regimens to be used in this protocol will increase this risk.

6.2 Dose Delays and Modifications

Because this is a transplantation regimen and there is only 1 cycle of treatment pre-transplant, no dose delays or modifications are possible for the elements of the HCT conditioning regimen. Only the components of the GVHD prophylactic regimen may be reduced.

6.2.1 Tacrolimus. Tacrolimus will start on day +5 at dose of 1 mg a day on a continuous infusion. This will be switched to equivalent oral dose at twice a day at the time of discharge and continue until day +180 post HCT. Tacrolimus taper will start at 25% on every 1 weeks bases when no evidence of GvHD. Earlier taper us allowed in patient with high risk disease.

6.2.2 Mycophenolate Mofetil MMF will be started on day +5 at 1gram IV three times a day and will be switched to oral when ready to be discharged. This will be stopped at day +35 post HCT, if no evidence of active GvHD.

7.0 Data and Safety Monitoring, Unanticipated Problems and Adverse Event Reporting

7.1 Risk Level

This is a Risk Level 3 study, as defined in the "City of Hope Data and Safety Monitoring Plan", http://www.coh.org/dsmc/Pages/forms-and-procedures.aspx involving COH as IND holder.

7.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the PI, Collaborating Investigators, CRC/protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of the stopping rules for safety and efficacy.

This study will utilize the Phase I tracking log to monitor data and safety for dose escalation, recording doses administered, and resultant adverse events**.** The tracking log will contain dose levels administered, DLT-defining adverse events, and documentation that the data from a dose level is complete before dose escalation. Those data and safety elements will be reported to the COH DSMC as applicable within the PMT report, which will be submitted quarterly from the anniversary date of activation.

7.3 Definitions

Adverse event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Serious Adverse Event (SAE) [21 CFR 312.32] - defined as any expected or unexpected adverse event that results in any of the following outcomes:

- Death
- Is life-threatening experiences (places the subject at immediate risk of death from the event as it occurred)

- Unplanned hospitalization equal or greater than 24 hours)) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Unanticipated problem (UP) - Any incident, experience or outcome that **meets all three** of the following criteria:

- Unexpected (in term nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; AND
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); AND
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

7.4 Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems - Most unanticipated problems must be reported to the COH DSMC and IRB **within 5 calendar days** according to definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf. Any unanticipated problem that occurs during the study conduct will be reported to the DSMC and IRB by submitting electronically in iRIS (http://iris.coh.org).

Serious Adverse Events - All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf and Table 2 below. Those SAEs that require expedited reporting will be submitted electronically in iRIS (http://iris.coh.org).

Adverse Events - Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of *serious* OR are not unanticipated problems will be reported only in the continuation reports and PMT reports.

8.0 Agent Information

8.1 Busulfan

8.1.1 Description/Pharmacology

Busulfan (1, 4-dimethanesulfonoxybutane) is an alkylating agent. The drug is extensively metabolized and its metabolites are eventually excreted in the urine. The oral preparation is

well absorbed but studies have indicated that there is a ten-fold variability area under the curve (AUC) of the drug among patients receiving busulfan by mouth. There is a statistical association between increased AUC and the development of VOD of the liver. Since its FDA approval in 1999, IV Bu has been used increasingly in combination with CY or Flu. IV Bu was initially administered every 6-hours, similar to oral Bu. However, several studies have used the drug with once or twice daily administration. In terms of safety, IV Bu and oral Bu appear to have similar toxicity profiles. It has been proposed that VOD and mucositis may be reduced in incidence and severity with IV Bu. The IV formulation at a dose of 0.8 mg/kg IV every 6-hrs is considered equivalent to the oral formulation at a dose of 1 mg/kg PO every 6 hrs. in conditioning regimens. On this basis a regimen using 4x0.8 = 3.2 mg/kg as a single daily dose has been developed.

8.1.2 Toxicology

Toxicities associated with busulfan administration include:

- Gastrointestinal: nausea, vomiting, constipation, diarrhea, abdominal discomfort, anorexia, dyspepsia and mucositis
- Hepatobiliary: Veno-occlusive disease
- Neurologic: headache, insomnia and seizures
- Cardiovascular: hypertension, hypotension and tachycardia
- Pulmonary: dyspnea, lung fibrosis
- Endocrine and metabolic: hypermagnesemia, hyperglycemia and hyperphosphatemia
- Miscellaneous: rhinorrhea, amenorrhea, infertility, skin rashes, cataracts

8.2 Cyclophosphamide

8.2.1 Description/Pharmacology

Cyclophosphamide (CY) is an alkylating agent which prevents cell division primarily by cross-linking DNA strands. CY is converted to its active form in vivo by hepatic enzymes. After a single dose, tissue enzymes degrade most of the active metabolites. After high doses (> 40 mg/kg), the alkylating activity in the plasma is minimal by 24 hours. Several of the metabolites appear to have toxic actions. One of the metabolic products, acrolein (CH2=CH-CHO), is known to be toxic to the bladder urothelium and can cause hemorrhagic cystitis when CY is administered at high doses.

8.2.1 Toxicology

Some of the most common toxicities associated with cyclophosphamide include:

- Gastrointestinal: nausea, vomiting and anorexia
- Hematologic: myelosuppression
- Cardiovascular: severe chronic heart failure characterized by cardiomegaly, pericardial effusions, diffuse voltage decrease on ECG and decreased LVEF
- Genitourinary: hemorrhagic cystitis (prevented by hydration and mesna therapy or bladder irrigation) and gonadal function impairment
- Miscellaneous: fluid retention, alopecia and rare pulmonary toxicity

8.3 Fludarabine

8.3.1 Description/Pharmacology

Fludarabine is a fluorinated nucleoside analog. After phosphorylation to fluoro-ara-ATP the drug appears to incorporate into DNA and inhibit DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. Excretion of fludarabine is impaired in patients with impaired renal function.

8.3.1 Toxicology

Some of the most common side effects of fludarabine include:

- Hematologic: hematopoietic suppression including neutropenia and lymphopenia with increased risk of infection and immunosuppression
- Neurologic: peripheral neuropathy and encephalopathy manifested by fatigue, weakness, paresthesia, visual disturbances, somnolence and coma
- Gastrointestinal: nausea, vomiting, diarrhea and stomatitis
- Miscellaneous: fever, skin rash, cough and idiopathic pneumonitis

8.4 Melphalan

8.4.1 Description/Pharmacology

Melphalan, also known as L-phenylalanine mustard, phenylalanine mustard, L-PAM, or L-sarcolysin, is a phenylalanine derivative of nitrogen mustard. Melphalan is a bifunctional alkylating agent that is active against selected human neoplastic diseases. It is known chemically as 4-[bis (2-chloroethyl) amino]-L-phenylalanine. The pharmacokinetics of melphalan after IV administration has been extensively studied in adult patients. Following injection, drug plasma concentrations declined rapidly in a biexponential manner with distribution phase and terminal elimination phase half-lives of approximately 10 and 75 minutes, respectively.

8.4.2 Reconstitution/Administration

Refer to institutional standards or prescribing information for preparation and administration for melphalan.

8.4.3 Toxicology

Common toxicities of melphalan include:

- Hematologic: bone marrow suppression and hemolytic anemia
- Gastrointestinal: severe stomatitis, mucositis, esophagitis and diarrhea
- Pulmonary: pulmonary fibrosis and interstitial pneumonitis
- Dermatologic: skin hypersensitivity and alopecia
- Miscellaneous: vasculitis and allergic reactions

8.5 Total Body Irradiation

8.5.1 Description/Pharmacology

TBI will be administered per standard of care procedure as implemented by radiation oncologists. TBI alone for post-pubescent patients with dose/fractionation of 1.5 Gy twice a

day for 4 day and total dose of 1200cGy within the tolerance of most normal organs for < 5% risk of severe late toxicity (organ failure or major dysfunction) by 5 years.

8.5.2 Toxicology

Common toxicities of total body irradiation include:

- Gastrointestinal: mucositis, nausea, vomiting and diarrhea
- Hematologic: marrow suppression
- Dermatologic: reversible skin pigmentation and alopecia
- Late effects: cataract formation, growth retardation, pulmonary damage, carcinogenesis and sterilization
- Miscellaneous: fever and parotiditis

8.6 Tacrolimus

8.6.1 Description/Pharmacology

Tacrolimus often called by its original drug code name, FK-506, is a macrolide immunosuppressant that inhibits calcineurin (phosphatase 2B)-mediated T-cell activation by forming a complex with FK506 binding protein 12 (FKBP12).

8.6.1 Toxicology

Some of the most common toxicities associated with Tacrolimus include:

- Cardiovascular: hypertension
- Neurologic: confusion, dizziness, insomnia, seizures, tremors, changes in how clearly one can think
- Gastrointestinal: nausea, vomiting
- Hematologic: microangiopathic hemolytic anemia, thrombocytopenia
- Endocrine and metabolic: hypomagnesemia, hypokalemia, hypocalcemia, hyperlipidemia
- Miscellaneous: unwanted hair growth, changes in vision, liver problems, reversible renal insufficiency, infections and post-transplant lymphoproliferative disorders

8.7 Mycophenolate mofetil (MMF)

8.7.1 Description/Pharmacology

Mycophenolate mofetil, a morpholinoethyl ester of mycophenolic acid antibiotic with immunosuppressant properties, is indicated for the prophylaxis of organ rejection in patients receiving allogeneic renal transplants. This product should be used concomitantly with cyclosporine and corticosteroids

8.7.2 Toxicology

Some of the most common toxicities associated with MMF include:

- Neurologic: headache, tremors, insomnia, dizziness, excessive fatigue, weakness
- Cardiovascular: tachycardia
- Pulmonary: dyspnea

- Gastrointestinal: nausea, vomiting, dyspepsia, abdominal pain, diarrhea, hematemesis and hematochezia
- Hematologic: Neutropenia, thrombocytopenia, unusual bruising, and anemia
- Endocrine and metabolic: hyperlipidemia
- Miscellaneous: rash, edema, change in vision, infection, second cancers, teratogenicity, miscarriage, limited effectiveness of birth control, and progressive multifocal leukoencephalopathy (PML).

8.8 MESNA (sodium -2-mercapto ethane-sulphonate)

8.8.1 Description/Pharmacology

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazophosphorines (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein; the urotoxic metabolite produced by the oxazophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazophosphorines.

8.8.1 Toxicology

At the doses used for uroprotection, mesna is virtually non-toxic. The most common side effects of MESNA are:

- Cardiovascular: hypotension
- Dermatologic: rash, urticarial
- Gastrointestinal: nausea and vomiting, diarrhea, abdominal pain, altered taste
- Neurologic: headache, joint or limb pain
- Miscellaneous: fatigue

9.0 Correlative/Special Studies

Immune correlative studies will be performed at the Fox South 1st floor (Nakamura). For the cellular assays below (#1-3), 30 ml blood samples in heparin Na tubes (green top) will be collected on days -9 (between days -30 and -9 as pre-HCT), and post-transplant on days 7, 14, 21, 28 (± 2 days), days 42-63 (±7 days), days 100 and 180 (± 14 days), 1 year (+/-30days), and 2 years (± 30 days) post-transplant. When available and with NMDP approval, we also plan to obtain 30 ml blood samples from the unrelated donors. Peripheral blood mononuclear cells (PBMC) will be separated using Ficoll and cryopreserved for further experiments as below (#1-3). For plasma cytokine/GVHD biomarker assays (#3 below), 10 ml of blood will be collected in lavender top tubes (K2 EDTA) and frozen on days +7, +14, +21, + 28, +35, +42, +63, +100, +180, and +365 (total 90ml) (see study calendar for the window).

1) Reconstitution of T cells, NK cells, and B cells: Standard flow-cytometry analyses will be performed to enumerate T cell subsets (i.e. effector, memory, naive T cells based on CD45RA/CD62L, exhaustion markers such as PD1, and Treg), B cell subsets (naïve/memory based on CD27, Breg[49]), NK subsets (i.e. memory NK with CD3-CD56+/NKG2C+ and other functional markers such as CD137)[50].

2) Functional characterization of T cells recovering post-HCT: Using a CD137 expression assay[51], we will enumerate CD4 and CD8 T cells capable of responding to specific antigens including CMV (pp65Ag), EBV (transformed LCL), VZV antigen, and leukemia antigens (i.e. WT1)[52,53] at each time point. When possible, CD137+ cells responding to the antigen stimulation will be sorted and subjected for T cell receptor (TCR) CDR3 region sequencing by NGS.

3) Plasma cytokines and GVHD biomarkers: Inflammatory cytokines (i.e. CRP, β-2 microglobulin, IL-6, and TNF-α) and GVHD biomarkers (i.e. IL2R, TNFR1, HGF, ST2, REG3a, Elafin)[54,55] will be assessed using the Luminex X-MAP bead array-based assay or standard ELISA where applicable.

10.0 Study Calendars

10.1 Study Assessment Calendar

Study Assessments (All Regimens)	Pre-HCT	Days post stem cell infusion														
		7 ±2	14 ±2	21 ±2	28 ±2	35 ± 7	42 ± 7	49 ± 7	56 ± 7	63 ± 7	100 ± 14	150 ± 14	180 ± 14	270 ± 14	365 ± 30	548 ± 30	730 ± 30
History, physical exam, weight and height[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Karnofsky or Lansky performance status	X				X						X		X		X	X	X
HCT-Specific Co-Morbidity Index (HCT-CI) score	X																
HLA typing (recipient and donor)	X																
CBC[2], differential, platelet count, and chemistries[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Measured creatinine clearance[4]	X															X	
Infectious disease markers[5]	X																
Cardiac assessments[6]	X															X	
Pulmonary function tests[7]	X												X			X	X
Disease evaluation[8]	X										X		X		X		
Chest x-ray or chest CT or CT/PET	X															X	
Pregnancy test[9]	X																
GVHD assessments[10]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessments[11]					X				X		X		X		X	X	X
CMV Monitoring		X	X	X	X	X	X	X	X	X	X	X	X	X			
EBV Monitoring		X	X	X	X	X	X	X	X	X	X	X	X	X			
Quality of Life assessments[12]	X										X		X			X	X
Research Samples[13]	X	X	X	X	X		X		X		X		X		X		X

[1]Height is only required at the Pre-HCT Baseline visit.

[2]CBC and manual WBC differential per SOP at COH

[3]Blood chemistries include: serum creatinine, bilirubin, alkaline phosphatase, AST and ALT. Blood chemistries performed twice weekly until hospital discharge. Blood chemistries performed weekly after hospital discharge until Day 63 post-transplant, then every other week through Day 100 post-transplant, and then at Days 180, 270 and 365 post-transplant.

[4]Measured creatinine clearance. The updated Schwartz formula should be used for pediatric patients (≥ 5 year to 12 years).

[5]Infectious disease markers include: CMV, Hepatitis panel (Hep B S Ag, Hep B Core Ab, Hep C Ab), herpes simplex virus, syphilis, HIV-1 and -2 and HTLV-I and -II antibody, and toxoplasmosis. Patient can move forward to transplant with pending results of added infectious disease labs for the following: HTLV 1 & 2 and toxoplasmosis.

[6]Cardiac assessments include EKG and left ventricular ejection fraction (LVEF) or shortening fraction by echocardiogram or radionuclide scan (MUGA).

[7]Pulmonary function tests include DLCO (Adj. for Hgb), FEV1 and FVC. For children who are unable to perform for PFTs due to age or developmental ability, there must be no evidence of dyspnea at rest and no need for supplemental oxygen (as evidenced by O_2 saturation > 92% on room air) at baseline in order to meet eligibility criteria.

[8]Disease evaluation: : BM aspirate and biopsy to be done within 30 days of start of conditioning for the following disease: Acute Leukemia: MDS; CLL/small lymphocytic lymphoma, follicular marginal zone, diffuse large B-cell, Hodgkin Lymphoma; mantle cell lymphoma with chemo sensitive disease at the time of transplantation. Myeloproliferative neoplasm (included MF). For patients with active disease BM aspirate and biopsy need to be done within 14 days of start of conditioning. Active diseases should be verified by the MD. Study PI will decide if a patient with active disease is allowed to move forward without having a BM aspirate and biopsy within 14 days of transplant. For leukemia patients, a bone marrow biopsy is required for disease status evaluation. For lymphoma patients, CT scans or whole body CT/PET scan is required on days indicated on the study calendar. Brain/spine MRIs are allowed on the opinion of the PI. For multiple myeloma patients, serum studies, BM and bone surveys at all days indicated on the calendar.

[9]Pregnancy test must be performed ≤ 30 days before the start of the transplant conditioning regimen. Pregnancy test is required for females of childbearing potential (i.e., not postmenopausal or surgically sterile), and may be performed per institutional practices.

[10]GVHD assessments performed weekly until Day 63 post-transplant, and then at Days 100, 150, 180, 270, 365, and 730. The GVHD assessment will include a review of **all** abnormalities experienced **during the entire assessment period** and the **highest grade** for each abnormality (*whether attributed to GVHD or not)*. aGVHD assessment will be done per 1994 Keystone consensus criteria and cGVHD assessment (Appendix B) will be done per NIH consensus staging (Appendix C).

[11]The toxicity assessment will include a review of **all** toxicities experienced **during the entire assessment period** and the **highest grade** for each toxicity will be recorded from Day -9 through Day 30. The highest grade of toxicities that meet grade 3, 4, & 5 will be recorded from Day 31-100.

[12]QOL assessments include self-reported patient questionnaires: SF-36, FACT-BMT, and MDASI for English and Spanish speaking patients > 18 years, and PedsQL Stem Cell Transplant Module for English speaking pediatric patients (ages 8 through 18 years).

[13]Research Sample collection for patients who weigh > 30.0 kg.The Baseline sample will be collected prior to initiation of the conditioning regimen.

10.2 Conditioning and GVHD regimens

Regimens (A, B, or C)	-7*	-6*	-5*	-4*	-3*	-2*	-1*	0*	+3*	+4*	+5*	+6*	+7*	+35	+90
Regimen A only (Flu/Mel Reduced Intensity)															
Hospital Admission	X														
Fludarabine (25 mg/m^2 daily)***	X	X	X	X	X										
Melphalan (140 mg/m^2) (or 100 mg/m^2 if age ≥60)						X									
HSC infusion (PBSC or BM)**[1]								X							
Cyclophosphamide (50 mg/kg daily)									X	X					
G-CSF 5 µg/kg daily (until ANC>1500)											X	X	X		
Tacrolimus 1 mg daily starting dose[2],											X----------X				
Mycophenolate Mofetil (MMF)[3]											X----------X				
Regimen B only (Flu/Bu Myeloablative)															
Hospital Admission		X													
Fludarabine (40 mg/m^2 daily – over 1 hr)			X	X	X	X									
Busulfan (130 mg/m^2 daily – over 1 hr)			X	X	X	X									
HSC infusion (PBSC or BM)[1]								X							
Cyclophosphamide (50 mg/kg daily)									X	X					
G-CSF 5 µg/kg daily (until ANC>1500)											X	X	X		
Tacrolimus 1 mg daily starting dose[2],											X----------X				
Mycophenolate Mofetil (MMF)[3]											X----------X				
Regimen C only (Flu/TBI Myeloablative)															
Hospital Admission	X														
Fludarabine (30 mg/m^2 daily)	X	X	X												
Total Body Irradiation 1200 cGy (150 cGy 2x daily)				X	X	X	X								
HSC infusion (PBSC or BM)[1]								X							
Cyclophosphamide (50 mg/kg daily)									X	X					
G-CSF 5 µg/kg daily (until ANC>1500)											X	X	X		
Tacrolimus 1 mg daily starting dose[2],											X----------X				
Mycophenolate Mofetil (MMF) [3]											X----------X				

* A window of 1-2 days is allowed for stem cell availability.

** Cryopreserved HSCs are allowed in the opinion of study PI.

***Fludarabine can be given out-patient in the opinion of study PI

1. HSC = hematopoietic stem cells, PBSC = peripheral blood stem cells, BM = bone marrow
2. Dose adjusted to keep blood levels between 5-15 ng/mL. Begin taper at ~+90 days and end by 180 if no acute GVHD.
3. 15 mg/kg PO TID (max 1g – stop at day +35 if no severe GVHD)

11.0 Data Reporting/Protocol Deviations

11.1 Data Reporting

11.1.1 Confidentiality and Storage of Records

The original data collection forms will be sent to in encrypted, password protected, secure computers/servers that meet HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

11.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

11.1.3 Data Collection Forms and Submission Schedule

All data will be collected 1-2 weeks using standard Medidata Electronic Data Capture (EDC) form. Data will be collected and stored on secure computers as indicated in Section 12.1.1. After 2 years, we will access the COH CIBMTR data repository to retrieve data regarding post – HCR long-term outcomes through study termination.

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

11.1.3.1 Prior Therapy Forms and On-Study Forms

Within 10 business days of registration, the clinical research associate will submit case report forms.

11.1.4 Data Forms Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 10 business days of registration
Baseline Assessment Forms	Within 10 business days of registration
Treatment Forms	Within 10 business days of treatment administration
Adverse Event Report Forms	Within 5 business days, but only through day 60 (DLT period)
Response Assessment Forms	Within 10 business days of response assessment
Other assessment forms (e.g. concomitant meds, chemistry, hematology, neuro exam, physical	Within 10 business days of the assessment

exam)	
Off Treatment/Off Study Forms	Within 10 business days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 10 business days of the protocol defined follow up visit date or call

11.2 Protocol Deviations

11.2.1 Deviation Policy

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation Policy" located at http://www.coh.org/dsmc/Documents/Institutional%20Deviation%20Policy.pdf.

Deviations from the written protocol that could increase patient risk or alter protocol integrity require prior IRB approval of a single subject exception (SSE) request. In addition, if contractually obligated, the sponsor must also approve the deviation. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Examples include, but are not limited to: a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are considered to be deviations from the protocol. A deviation report will be submitted to the DSMC/IRB within five days.

11.2.2 Reporting of Deviations

All deviations will be reported to the COH DSMC within five days. The DSMC will forward to report to the IRB following review.

11.2.3 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol, it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair (or designee) to arrive at resolution.

12.0 Endpoint Evaluation Criteria/Measurement of Effect

12.1 Primary Endpoint

- GVHD-free Relapse/Progression-free Survival (GRFS): defined as time from start of treatment (HCT) to grade 3-4 acute GVHD, moderate-severe chronic GVHD, relapse, progression or death (from any cause), whichever occurs first. If a patient has not experienced any of these events, GRFS is censored at time of last follow-up.

12.2 Secondary Endpoints:

- Toxicity: Toxicities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 and the Bearman Toxicity Scale (for 100 days post-HCT)[48].

Note: The highest grade of all toxicities will be recorded from Day -9 to Day 30. The highest grade of toxicities that meet grade 3, 4, or 5 per CTCAE v4.03 from day +31 to +100 post-transplant will be collected. Start and stop dates will also be recorded for any grade 4 neutropenia.

- Unacceptable Toxicity: To be evaluable for toxicity, a patient must start conditioning and be observed for 30 days from stem cell infusion or have experienced an unacceptable toxicity. For the purposes of this study, unacceptable toxicity will be defined as any of the following that are assigned an attribution level of at least possibly related to the study regimen:
    - For non-hematologic toxicities, any regimen-related grade III/IV toxicity per Bearman Toxicity Grading Scale or
    - For non-hematologic toxicities (not part of the Bearman toxicity grading scale), any ≥grade 4 toxicity per NCI CTCAE v4.03, except for metabolic/electrolyte disturbances and vomiting controlled by medical management.
    - For hematologic toxicities, per NCI CTCAE v4.03 toxicity criteria, any grade 4 neutropenia associated with fever or infection and lasting for more than 21 days, or grade 4 neutropenia lasting for more than 28 days (engraftment failure) 42 days acceptable for myelofibrosis patients to achieve engraftment
    - Any other regimen-related cause of death.
- Infection: Microbiologically documented infections will be reported by site of disease, date of onset, severity and resolution, if any. These data will be captured via case report form and will be collected from day 0 until 100 days post-transplant.
- Acute Graft versus Host Disease (aGVHD) of grades 2-4 and 3-4: Acute graft versus host disease is graded according to the 1994 Keystone Consensus Grading[56] (Appendix B). The first day of acute GVHD onset at a certain grade will be used to calculate cumulative incidence curves for that GVHD grade; relapse/death prior to onset will be considered competing events. The endpoint will be evaluated from day 0 through 100 days post-transplant.
- Chronic Graft versus Host Disease (cGvHD): Chronic graft versus host disease is scored according to NIH Consensus Staging[57,58] (Appendix C). The first day of chronic GvHD onset will be used to calculate cumulative incidence curves, with relapse / death prior to onset considered competing events. The endpoint will be evaluated from day 100 to the onset of chronic GvHD, death or last contact, whichever comes first.
- Engraftment: Time to hematological recovery in terms of neutrophil (ANC≥500/μL, $1.0x10^3$/μL) and platelet ($20x10^3$/μL and $100x10^3$/μL) engraftment time.

- Overall survival (OS): Patients are considered a failure for this endpoint if they die, regardless of cause. Time to this event is the time from start of protocol therapy to death, or last follow-up, whichever comes first.
- Progression-free survival (PFS): Patients are considered a failure for this endpoint if they relapse/progress or die, regardless of cause. Time to this event is the time from start of protocol therapy to death, relapse/progression, or last follow-up, whichever comes first.
- Relapse/Progression (CIR): The event is relapse/progression. Time to this event is measured from start of therapy. Death without relapse/progression is considered a competing risk. Surviving patients with no history of relapse/ progression are censored at time of last follow-up.
- Non-relapse Mortality (NRM): Patients are considered a failure for this endpoint if they die from causes other than relapse or progression. NRM is measured from start of therapy until non-disease related death, or last follow-up, whichever comes first.
- Quality of Life (QOL): assessments include self-reported patient questionnaires: SF-36, FACT-BMT, and MDASI for English and Spanish speaking patients > 18 years, and PedsQL Stem Cell Transplant Module for English speaking pediatric patients (ages 8 through 18 years).

12.3 Response Criteria

Response criteria will be specific to each disease with only the following categories being relevant post-transplant:

a. Complete remission
b. Relapse
c. Disease persistence/progression

For detailed description of the corresponding categories within the response criteria for each disease please check the following references:

ALL: NCCN guidelines for ALL Version 3.2017

a. Complete remission:
   - No circulating blasts or extramedullary disease
     - No lymphadenopathy, splenomegaly, skin/gum infiltration/testicular mass/CNS involvement
   - Trilineage hematopoiesis (TLH) and 5% blast
   - Absolute neutrophil count (ANC)>1000/microL
   - Platelets>100,000/microL
   - No recurrence for 4 weeks
b. Relapse
   - Reappearance of blasts in the blood or BM (>5%) or in any extramedullary site after CR
c. Disease persistence/progression
   - Failure to achieve CR at the end of induction
   - Increase of at least 25% in the absolute number of circulating or BM blasts or development of extramedullary disease.

AML: Döhner, Estey et al. 2017[59]

a. Complete remission:
   - CR without minimal residual disease (CR_{MRD-}):
     - If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC
   - Complete remission (CR):
     - Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100 000/μL)
   - CR with incomplete hematologic recovery (CR_i):
     - All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100 000/μL])

b. Relapse:
   - Hematologic relapse (after CR_{MRD-}, CR, CR_i):
     - Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease
   - Molecular relapse (after CR_{MRD-}):
     - If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC

c. Disease persistence/progression
   - Primary refractory disease:
     - No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause.
   - Progressive disease (PD):
     - Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood:
     - >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 mo; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/μL], and/or platelet count to >50 × 10^9/L [50 000/μL] nontransfused); or,
     - >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome); or
     - New extramedullary disease

MDS: Savona, Malcovati et al. 2015[60]

a. Complete Remission**(presence of all of the following improvements)**
   - Bone marrow:
     - ≤5% myeloblasts (including monocytic blast equivalent in case of CMML) with normal maturation of all cell lines and return to normal cellularity
     - Osteomyelofibrosis absent or equal to “mild reticulin fibrosis” (≤grade 1 fibrosis)
   - Peripheral blood
     - WBC ≤10 × 10^9 cells/L
     - Hgb ≥11 g/dL
     - Platelets ≥100 × 10^9/L; ≤450 × 10^9/L
     - Neutrophils ≥1.0 × 10^9/L
     - Blasts 0%
     - Neutrophil precursors reduced to ≤ 2%
     - Monocytes ≤1 × 10^9/L
   - Extramedullary disease: Complete resolution of extramedullary disease present before therapy (eg, cutaneous disease, disease-related serous effusions), including palpable hepatosplenomegaly
   - Provisional category of CR with resolution of symptoms: CR as described above, and complete resolution of disease-related symptoms as noted by the MPN-SAF TSS

- Persistent low-level dysplasia is permitted given subjectivity of assignment of dysplasia

b. Relapse (Partial Remission)
   - Normalization of peripheral counts and hepatosplenomegaly with bone marrow blasts (and blast equivalents) reduced by 50%, but remaining >5% of cellularity *except* in cases of MDS/MPN with ≤5% bone marrow blasts at baseline.
   - **Marrow response**
     - Optimal marrow response: Presence of all marrow criteria necessary for CR without normalization of peripheral blood indices as presented above.
     - Partial marrow response: Bone marrow blasts (and blast equivalents) reduced by 50%, but remaining >5% of cellularity, *or* reduction in grading of reticulin fibrosis from baseline on at least 2 bone marrow evaluations spaced at least 2 mo apart

c. Disease persistence/progression: (Combination of 2 major criteria, 1 major and 2 minor criteria, or 3 minor criteria from list)
   - Major criteria
     - Increase in blast count*
       - <5% blasts: ≥50% increase and to >5% blasts
       - 5-10% blasts: ≥50% increase and to >10% blasts
       - 10-20% blasts: ≥50% increase and to >20% blasts
       - 20-30% blasts: ≥50% increase and to >30% blasts
     - Evidence of cytogenetic evolution
       - Appearance of a previously present or new cytogenetic abnormality in complete cytogenetic remission via FISH or classic karyotyping
       - Increase in cytogenetic burden of disease by ≥50% in partial cytogenetic remission via FISH or classic karyotyping
     - New extramedullary disease
       - Worsening splenomegaly
       - Progressive splenomegaly that is defined by IWG-MRT: the appearance of a previously absent splenomegaly that is palpable at >5 cm below the left costal margin or a minimum 100% increase in palpable distance for baseline splenomegaly of 5-10 cm or a minimum 50% increase in palpable distance for baseline splenomegaly of >10 cm
       - Extramedullary disease outside of the spleen
       - To include new/worsening hepatomegaly, granulocytic sarcoma, skin lesions, etc.
   - Minor criteria
     - Transfusion dependence§
     - Significant loss of maximal response on cytopenias ≥50% decrement from maximum remission/response in granulocytes or platelets
     - Reduction in Hgb by ≥1.5g/dL from best response or from baseline as noted on complete blood count
     - Increasing symptoms as noted by increase in ≥50% as per the MPN-SAF TSS||
     - Evidence of clonal evolution (molecular)

Myelofibrosis: Tefferi, Cervantes et al. 2013[61] and Zang and Deeg 2009[62]

a. Complete Remission:
   - Bone marrow: Age-adjusted normocellularity; <5% blasts; ≤grade 1 MF and,
   - Peripheral blood: Hemoglobin ≥100 g/L and <UNL; neutrophil count ≥ 1 × 109/L and <UNL;
   - Platelet count ≥100 × 109/L and <UNL; <2% immature myeloid cells and

- Clinical: Resolution of disease symptoms; spleen and liver not palpable; no evidence of EMH

b. Relapse:
- No longer meeting criteria for at least CI after achieving CR, PR, or CI, or
- Loss of anemia response persisting for at least 1 month or
- Loss of spleen response persisting for at least 1 month
- Recommendations for assessing treatment-induced cytogenetic and molecular changes

c. Disease persistence/progression:
- Appearance of a new splenomegaly that is palpable at least 5 cm below the LCM or
- A ≥100% increase in palpable distance, below LCM, for baseline splenomegaly of 5-10 cm or
- A 50% increase in palpable distance, below LCM, for baseline splenomegaly of >10 cm or
- Leukemic transformation confirmed by a bone marrow blast count of ≥20% or
- A peripheral blood blast content of ≥20% associated with an absolute blast count of ≥1 × 10(9)/L that lasts for at least 2 weeks

CLL: NCCN guidelines for CLL, Version 1.2018

a. Complete remission:
- Group A Lymphadenopathy: None > 1.5 cm
- No hepatomegaly
- No splenomegaly
- Marrow:
  - Normocellular
  - <30%Lymphocytes
  - No B-lymphoid nodules
  - Hypercellular marrow defines CR with incomplete marrow recovery
- Blood lymphocyte<4000/micro/L
- Group B Platelet count without growth factors>100,000/micro/L
- Hb without transfusions or growth factors>11.0 g/dL
- Neutrophils without growth factors>1500/micro/L

b. Relapse (partial Remission)
- Group A Lymphadenopathy: decrease≥50%
- Hepatomegaly≥50%
- Splenomegaly≥50%
- Marrow: 50% reduction in marrow infiltrate, or B-lymphoid nodules
- Blood lymphocyte: Increase or decrease <50% over baseline
- Group B Platelet count without growth factors>100,000/micro/L or increase ≥50% over baseline
- Hb without transfusions or growth factors>11.0 g/dL or increase ≥50%overbaseline
- Neutrophils without growth factors>1500/micro/L or >50% improvement over baseline

c. Disease persistence/progression:
- Group A Lymphadenopathy: Increase ≥50%
- Hepatomegaly: Increase ≥50%
- Splenomegaly: Increase ≥50%
- Blood lymphocyte: Increase ≥50% over baseline
- Group B Platelet count without growth factors: Decrease ≥50% over baseline
- Hb without transfusions or growth factors: Decrease of >g/dL from baseline secondary to CLL

Hodgkin and non-Hodgkin Lymphoma: Cheson, Fisher et al. 2014[63]

a. Complete remission (CT-Based Response)
   - Lymph nodes and extralymphatic sites: No extralymphatic sites of disease
   - Nonmeasured lesion: Absent
   - Organ enlargement: Regress to normal
   - Lesions: None
   - Bone Marrow: Normal morphology, if indetereminate, IHC negative

b. Relapse (partial remission)
   - Lymph nodes and extralymphatic sites: ≥ 50% decrease in SPD of up to 6 target measurable nodes and extranodal sites
   - Nonmeasured lesions:
     - When a lesion is too small to measure on CT, assign 5 mm × 5 mm as the default value
     - For a node > 5 mm × 5 mm, but smaller than normal, use actual measurement for calculation
   - Organ enlargement: Spleen must have regressed by > 50% in length beyond normal
   - Lesions: None
   - Bone marrow: Not applicable

c. Disease persistence/progression:
   - Target nodes/nodal masses, extranodal lesions: < 50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
   - Nonmeasured lesions: No increase consistent with progression
   - Organ enlargement: No increase consistent with progression
   - Lesions: None
   - Bone marrow: Not applicable

Progressive disease requires at least 1 of the following:

- Extranodal lesions:
  - An individual node/lesion must be abnormal with:
    - LDi > 1.5 cm and
    - Increase by ≥ 50% from PPD nadir and
    - An increase in LDi or SDi from nadir
    - 0.5 cm for lesions ≤ 2 cm
    - 1.0 cm for lesions > 2 cm
    - In the setting of splenomegaly, the splenic length must increase by > 50% of the extent of its prior increase beyond baseline (eg, a 15-cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline
      New or recurrent splenomegaly.
- Nonmeasured lesions: New or clear progression of preexisting nonmeasured lesions
- Lesions:
  - Regrowth of previously resolved lesions
  - A new node > 1.5 cm in any axis
  - A new extranodal site > 1.0 cm in any axis; if < 1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma
  - Assessable disease of any size unequivocally attributable to lymphoma
- Bone marrow: New or recurrent involvement

Multiple Myeloma: NCCN Guidelines for Multiple Myeloma Version 2.2018

a. Complete Response:
   - Negative immunofixation on the serum and urine and disappearance of any soft tissue plasma

b. Relapse: Clinical relapse require one or more of the following
- Direct indicators of increasing disease and/or end organ dysfunction (calcium elevation, renal failure, anemia, lytic bone lesion [CRAB features] related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but it is listed as something that can be reported optionally or for use in clinical practice;
- Development of new soft tissue or bone lesions (osteoporotic features do not constitute progression);
-

c. Disease persistence/progression:
- Stable Disease: not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates.
- Progressive disease: any one or more of the following criteria:
  - Increase of 25% from lowest confirmed response value in one or more of the following criteria:
    - Serum M-protein (absolute increase must be ≥0.5 g/dL;
    - Serum M-protein increase ≥ g/dL, if the lowest M component was ≥0.5 g/dL;
    - Urine M protein (absolute increase must be ≥200 mg/24 h);
    - In patients without measurable serum and urine M-protein levels, the differences between involved and uninvolved FLC levels (absolute increase must be>10 mg/dL);
    - In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%);
    - Appearance of a new lesion(s), ≥50% increase from nadir in SPD of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis;
    - ≥50%increase in circulating plasma cells (minimum of 200 cells per µl) if this is the only measure of disease.

CML: Oehler. 2013[64] and NCCN guidelines for CML version 1.2018

a. Complete response:
- Leukocyte count of <10 X 10^9/L
- Platelet count < 450 X 10^9/L
- Normal differential with no early forms
- No splenomegaly

b. Relapse:
- Any sign of loss of response (defined as hematologic or cytogenetic relapse)
- 1-log increase in BCR-ABL1 transcript levels with loss of MMR should rompt bone marrow evaluation for loss of CCyR but is not itself defined as relapse (eg, hematologic or cytogenetic relapse)

13.0 Statistical Considerations

13.1 Study Design

We will assess the clinical activity, overall and per stratum (RIC or MAC), and evaluate the safety of post-transplant high dose cyclophosphamide (PTCy) in patients undergoing T-cell replete HLA-mismatched unrelated donor hematopoietic cell transplantation (HCT) for

hematologic malignancy. The primary endpoint for this single center, pilot/estimation study is one-year GVHD, relapse/progression-free survival (GRFS).

13.2 Sample Size Accrual Rate

Due to a lack of comparable historical data/ estimates[65-67] for one-year GRFS in this setting, a total of 38 patients, with 19 patients per stratum is sufficient to estimate the GRFS rate at 1 year with adequate precision (standard error = .08 overall, = 0.12 per stratum (see the following table for detail). On average we perform 15-20 MMUD HCTs annually at City of Hope. (Note: There is no competing trial for MMUD HCT since most of the HCT trials avoid MMUD HCT.) We anticipate that the accrual of 38 patients will take 2 years to complete. With the primary endpoint of 1-yr GRFS, and up to 2-years of follow-up planned, the study is expected to complete in – approximately 4 years.

Censored	**Overall (N=38)**			**Per Stratum (N=19)**		
Before 1 yr	**1-yr GRFS rate**	**SE***	**95%CI***	**1-yr GRFS rate**	**SE***	**95%CI***
None	42%	8%	26% to 57%	42%	11%	20% to 62%
None	37%	8%	22% to 52%	37%	11%	17% to 57%
None	32%	8%	18% to 46%	32%	11%	13% to 52%
None	26%	7%	14% to 41%	26%	10%	10% to 47%
None	21%	7%	10% to 35%	21%	9%	7% to 41%
1 patient	41%	8%	25% to 56%	39%	12%	18% to 60%
1 patient	36%	8%	21% to 51%	34%	11%	14% to 55%
1 patient	30%	8%	16% to 45%	28%	11%	10% to 49%
1 patient	25%	7%	12% to 39%	22%	10%	7% to 43%
1 patient	19%	6%	8% to 33%			

* Standard error (SE) and confidence interval (CI).

13.3 Stopping Rules for Excessive Toxicity

The following table will be consulted as relevant toxicities are encountered. Within each stratum, the early stopping rule for safety/toxicity will be assessed for each patient at day +30 post-transplant/stem cell infusion. The expected rate of unacceptable toxicity (defined in 12.2) should not be ≥33%. See the table below for detailed early stopping rules. If the specified number of patients is met or exceeded, patient accrual will be halted and a full review of the data by the Data Safety Monitoring Committee will be mandated. Patient accrual will not resume until approved by the Data Safety Monitoring Committee to do so.

# of patients treated	# of patients with unacceptable toxicity to halt enrollment[1]	Cumulative probability of early stopping given a toxicity rate of:		
		15%	33%	45%
6	2	0.22	0.64	0.84
12	4	0.24	0.73	0.92
[1]: For each unacceptable toxicity, halt enrollment and evaluate if the cumulative # of patients reaches or exceeds the specified limits.				

13.3 Statistical Analysis Plan

Survival estimates will be calculated using the Kaplan-Meier method, Greenwood formula will be used to calculate SE, and log-log transformation method will be used to construct 95% CI. The cumulative incidence of relapse/progression and non-relapse mortality will be calculated as competing risks using the Gray method. Toxicity information recorded will include the type, severity, and the probable association with the study regimen. Tables will be constructed to summarize the observed incidence by severity and type of toxicity. Baseline information (e.g. the extent of prior therapy) and demographic information will be presented, to describe the patients treated in this study.

14.0 Human Subject Issues

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2 Recruitment of Subjects

Study candidates will be recruited from patients undergoing HCT (cancer treatment) at City of Hope Cancer Center for hematological malignancies. Attending physicians and transplant coordinators identify potential candidates. The PI and the protocol team will review these cases and coordinate with the treating MDs for the consenting and enrollment process.

14.3 Advertisements

Advertisements to include print, media (radio, television, billboards), telephone scripts, lay summary to be posted on City of Hope's public Clinical Trials On-Line ^{SM} website, etc., will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

14.4 Study location and Performance Sites

This study will be performed at COH.

14.5 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results

14.6 Financial Obligations and Compensation

Eligible diseases in this trial are approved indications for cyclophosphamide. Thus the study drug will be covered by the insurance carrier. Once approved it can be continued for these patients. For rare cases the insurance authorization and prescription will be obtained prior to the study treatment. Informed consent and enrollment may occur prior to the insurance authorization. Since myelofibrosis is the FDA-approved indication we do not expect any denial, and cyclophosphamide should be ready by the time of the study treatment. If there is a denial or delay for whatever reasons after enrollment (we expect this case to be <1%), the patient will be considered as not evaluable and will be replaced

The standard of care drug(s) and procedures (the 3 acceptable HCT conditioning regimens are and HCT procedures and supportive care) provided will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

14.7 Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

Before signing the study consent form, HIPAA authorization form and the Experimental Subject's Bill of Rights, research subjects will undergo an assessment of their comprehension of the study by the Research Subject Advocate. Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be made and the questionnaire repeated until a satisfactory result is obtained. Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of

discussion, education and proctoring will be ineligible for enrollment. For those subjects who do comprehend the fundamental aspects of the study, consent will be obtained and documented, followed by eligibility testing. The research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment.

References

1. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18(6):695-706.

2. Ferrara JL. Pathogenesis of acute graft-versus-host disease: cytokines and cellular effectors. *J Hematother Stem Cell Res*. 2000;9(3):299-306.

3. den Haan JM, Sherman NE, Blokland E, et al. Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science*. 1995;268(5216):1476-1480.

4. Goulmy E, Schipper R, Pool J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med*. 1996;334(5):281-285.

5. Simpson D. New developments in the prophylaxis and treatment of graft versus host disease. *Expert Opin Pharmacother*. 2001;2(7):1109-1117.

6. Champlin RE, Schmitz N, Horowitz MM, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). *Blood*. 2000;95(12):3702-3709.

7. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood*. 2010;115(13):2704-2708.

8. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110(13):4576-4583.

9. Eapen M, Logan BR, Confer DL, et al. Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. *Biol Blood Marrow Transplant*. 2007;13(12):1461-1468.

10. Woolfrey A, Klein JP, Haagenson M, et al. HLA-C antigen mismatch is associated with worse outcome in unrelated donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2011;17(6):885-892.

11. Ringden O, Pavletic SZ, Anasetti C, et al. The graft-versus-leukemia effect using matched unrelated donors is not superior to HLA-identical siblings for hematopoietic stem cell transplantation. *Blood*. 2009;113(13):3110-3118.

12. Shaw BE, Gooley TA, Malkki M, et al. The importance of HLA-DPB1 in unrelated donor hematopoietic cell transplantation. *Blood*. 2007;110(13):4560-4566.

13. Antin JH, Ferrara JL. Cytokine dysregulation and acute graft-versus-host disease. *Blood*. 1992;80(12):2964-2968.

14. Baan CC, van der Mast BJ, Klepper M, et al. Differential effect of calcineurin inhibitors, anti-CD25 antibodies and rapamycin on the induction of FOXP3 in human T cells. *Transplantation*. 2005;80(1):110-117.

15. Zorn E. CD4+CD25+ regulatory T cells in human hematopoietic cell transplantation. *Semin Cancer Biol*. 2006;16(2):150-159.

16. Rieger K, Loddenkemper C, Maul J, et al. Mucosal FOXP3+ regulatory T cells are numerically deficient in acute and chronic GvHD. *Blood*. 2006;107(4):1717-1723.

17. Weisdorf D, Haake R, Blazar B, et al. Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. *Blood*. 1990;75(4):1024-1030.

18. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. *Blood*. 1991;77(8):1821-1828.

19. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med*. 1986;314(12):729-735.

20. Fay JW, Wingard JR, Antin JH, et al. FK506 (Tacrolimus) monotherapy for prevention of graft-versus-host disease after histocompatible sibling allogenic bone marrow transplantation. *Blood*. 1996;87(8):3514-3519.

21. Przepiorka D, Devine S, Fay J, Uberti J, Wingard J. Practical considerations in the use of tacrolimus for allogeneic marrow transplantation. *Bone Marrow Transplant*. 1999;24(10):1053-1056.

22. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood*. 2000;96(6):2062-2068.

23. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. *N Engl J Med*. 2014;371(4):339-348.

24. Petersdorf EW, Gooley T, Malkki M, Horowitz M, International Histocompatibility Working Group in Hematopoietic Cell T. Clinical significance of donor-recipient HLA matching on survival after myeloablative hematopoietic cell transplantation from unrelated donors. *Tissue Antigens*. 2007;69 Suppl 1:25-30.

25. Verneris MR, Lee SJ, Ahn KW, et al. HLA Mismatch Is Associated with Worse Outcomes after Unrelated Donor Reduced-Intensity Conditioning Hematopoietic Cell Transplantation: An Analysis from the Center for International Blood and Marrow Transplant Research. *Biol Blood Marrow Transplant*. 2015;21(10):1783-1789.

26. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood*. 2004;104(7):1923-1930.

27. Cutler C, Logan B, Nakamura R, et al. Tacrolimus/sirolimus vs tacrolimus/methotrexate as GVHD prophylaxis after matched, related donor allogeneic HCT. *Blood*. 2014;124(8):1372-1377.

28. Pidala J, Kim J, Jim H, et al. A randomized phase II study to evaluate tacrolimus in combination with sirolimus or methotrexate after allogeneic hematopoietic cell transplantation. *Haematologica*. 2012;97(12):1882-1889.

29. Luznik L, Bolanos-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. *Blood*. 2010;115(16):3224-3230.

30. Luznik L, Fuchs EJ. High-dose, post-transplantation cyclophosphamide to promote graft-host tolerance after allogeneic hematopoietic stem cell transplantation. *Immunol Res*. 2010;47(1-3):65-77.

31. Luznik L, Jones RJ, Fuchs EJ. High-dose cyclophosphamide for graft-versus-host disease prevention. *Curr Opin Hematol*. 2010;17(6):493-499.

32. Brunstein CG, Fuchs EJ, Carter SL, et al. Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood*. 2011;118(2):282-288.

33. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641-650.

34. Bolanos-Meade J, Fuchs EJ, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood*. 2012;120(22):4285-4291.

35. Kanakry CG, Ganguly S, Zahurak M, et al. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. *Sci Transl Med*. 2013;5(211):211ra157.

36. Owens AH, Jr., Santos GW. The effect of cytotoxic drugs on graft-versus-host disease in mice. *Transplantation*. 1971;11(4):378-382.

37. Jones RJ, Barber JP, Vala MS, et al. Assessment of aldehyde dehydrogenase in viable cells. *Blood*. 1995;85(10):2742-2746.

38. Luznik L, Engstrom LW, Iannone R, Fuchs EJ. Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation. *Biol Blood Marrow Transplant*. 2002;8(3):131-138.

39. Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. *Blood*. 2001;98(12):3456-3464.

40. Ciurea SO, Mulanovich V, Saliba RM, et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2012;18(12):1835-1844.

41. Bashey A, Zhang X, Sizemore CA, et al. T-cell-replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. *J Clin Oncol*. 2013;31(10):1310-1316.

42. Solomon SR, Sizemore CA, Sanacore M, et al. Total Body Irradiation-Based Myeloablative Haploidentical Stem Cell Transplantation Is a Safe and Effective Alternative to Unrelated Donor Transplantation in Patients Without Matched Sibling Donors. *Biol Blood Marrow Transplant*. 2015;21(7):1299-1307.

43. Kanakry CG, O'Donnell PV, Furlong T, et al. Multi-institutional study of post-transplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. *J Clin Oncol*. 2014;32(31):3497-3505.

44. Solomon SR, Sanacore M, Zhang X, et al. Calcineurin inhibitor--free graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide and brief-course sirolimus following reduced-intensity peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2014;20(11):1828-1834.

45. Al-Homsi AS, Cole K, Bogema M, Duffner U, Williams S, Mageed A. Short Course of Post-Transplantation Cyclophosphamide and Bortezomib for Graft-versus-Host Disease Prevention after Allogeneic Peripheral Blood Stem Cell Transplantation Is Feasible and Yields Favorable Results: A Phase I Study. *Biol Blood Marrow Transplant*. 2015;21(7):1315-1320.

46. Kanakry JA, Kasamon YL, Bolanos-Meade J, et al. Absence of post-transplantation lymphoproliferative disorder after allogeneic blood or marrow transplantation using post-transplantation cyclophosphamide as graft-versus-host disease prophylaxis. *Biol Blood Marrow Transplant*. 2013;19(10):1514-1517.

47. Di Stasi A, Milton DR, Poon LM, et al. Similar transplantation outcomes for acute myeloid leukemia and myelodysplastic syndrome patients with haploidentical versus 10/10 human leukocyte antigen-matched unrelated and related donors. *Biol Blood Marrow Transplant*. 2014;20(12):1975-1981.

48. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol*. 1988;6(10):1562-1568.

49. Khoder A, Sarvaria A, Alsuliman A, et al. Regulatory B cells are enriched within the IgM memory and transitional subsets in healthy donors but are deficient in chronic GVHD. *Blood*. 2014;124(13):2034-2045.

50. Foley B, Felices M, Cichocki F, Cooley S, Verneris MR, Miller JS. The biology of NK cells and their receptors affects clinical outcomes after hematopoietic cell transplantation (HCT). *Immunol Rev*. 2014;258(1):45-63.

51. Nakamura R, La Rosa C, Tsai W, et al. Ex vivo detection of CD8 T cells specific for H-Y minor histocompatibility antigens in allogeneic hematopoietic stem cell transplant recipients. *Transpl Immunol*. 2014;30(4):128-135.

52. Bleakley M, Heimfeld S, Jones LA, Turtle C, Riddell SR, Shlomchik W. Engineering Human Peripheral Blood Stem Cell Grafts That Are Depleted of Naïve T Cells and Retain Functional Pathogen-Specific Memory T cells. *Biol Blood Marrow Transplant*. 2014.

53. Israyelyan A, La Rosa C, Tsai W, et al. Detection and preliminary characterization of CD8+T lymphocytes specific for Wilms' tumor antigen in patients with non-Hodgkin lymphoma. *Leuk Lymphoma*. 2013;54(11):2490-2499.

54. Levine JE, Braun TM, Harris AC, et al. A prognostic score for acute graft-versus-host disease based on biomarkers: a multicentre study. *Lancet Haematol*. 2015;2(1):e21-29.

55. Vander Lugt MT, Braun TM, Hanash S, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. *N Engl J Med*. 2013;369(6):529-539.

56. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15(6):825-828.

57. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11(12):945-956.

58. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21(3):389-401 e381.

59. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.

60. Savona MR, Malcovati L, Komrokji R, et al. An international consortium proposal of uniform response criteria for myelodysplastic/myeloproliferative neoplasms (MDS/MPN) in adults. *Blood*. 2015;125(12):1857-1865.

61. Tefferi A, Cervantes F, Mesa R, et al. Revised response criteria for myelofibrosis: International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus report. *Blood*. 2013;122(8):1395-1398.

62. Zang DY, Deeg HJ. Allogeneic hematopoietic cell transplantation for patients with myelofibrosis. *Curr Opin Hematol*. 2009;16(2):140-146.

63. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059-3068.

64. Oehler VG. Update on current monitoring recommendations in chronic myeloid leukemia: practical points for clinical practice. *Hematology Am Soc Hematol Educ Program*. 2013;2013:176-183.

65. Holtan SG, DeFor TE, Lazaryan A, et al. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood*. 2015;125(8):1333-1338.

66. Solh M, Zhang X, Connor K, et al. Factors Predicting Graft-versus-Host Disease-Free, Relapse-Free Survival after Allogeneic Hematopoietic Cell Transplantation: Multivariable Analysis from a Single Center. *Biol Blood Marrow Transplant*. 2016;22(8):1403-1409.

67. Konuma T, Kato S, Oiwa-Monna M, Ishii H, Tojo A, Takahashi S. Comparison of graft-versus-host disease-free, relapse-free survival of transplantation using matched sibling donor, matched unrelated donor or unrelated cord blood after myeloablative conditioning for adult patients with hematological malignancies. *Leuk Lymphoma*. 2016;57(9):2126-2132.

68. Karnofsky DA, Abelmann WH, Craver LF, Burchenal JH. The use of the nitrogen mustards in the palliative treatment of carcinoma. With particular reference to bronchogenic carcinoma. *Cancer*. 1948;1(4):634-656.

15.0 Appendix

15.1 Appendix A: Comorbidity Index Comorbidity Index

IRB 16419

HCT-SPECIFIC COMORBIDITY INDEX SCORE

Comorbidities	Definition	Score
Migraine/headache		0
Osteoporosis		0
Osteoarthritis		0
Hypertension		0
Gastrointestinal	Including inflammatory bowel disease	0
Mild pulmonary	DLCo and/or FEV_1 >80% or Dyspnea on moderate activity	0
Mild renal	Serum creatinine 1.2-2 mg/dl	0
Endocrine		0
Bleeding		0
Coagulopathy	Deep venous thrombosis or pulmonary embolism	0
Asthma		0
Arrhythmia		1
Myocardial	Coronary artery disease, congestive HF, history of medically documented MI, EF≤50%	1
Mild hepatic	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN	1
Cerebro-vascular accident	History of transient ischemic attack or cerebro-vascular accident	1
Morbid obesity		1
Diabetes	Requiring treatment	1
Depression/anxiety		1
Infection	Requiring continuation of treatment after Day 0	1
Rheumatologic	SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica	2
Moderate pulmonary	DLCo and/or FEV_1 66-80% or Dyspnea on slight activity	2
Peptic ulcer	Patients who have required treatment	2
Moderate-severe renal	Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation	2
Valvular heart disease	Except mitral valve prolapse	3
Prior solid tumor	Requiring treatment with chemotherapy	3
Moderate-severe hepatic	Liver cirrhosis, Bilirubin >1.5 X ULN, or AST/ALT >2.5XULN	3
Severe pulmonary	DLCo and/or FEV_1 ≤65% or Dyspnea at rest or requiring oxygen	3

Total score is the sum of all comorbidities present pre-transplant.

Physician Signature_____ Date_____

15.2 Appendix B: Acute GVHD grading

1994 Keystone Consensus Criteria
Organ Staging of Clinical Acute GVHD

Skin	Lower GI	Upper GI	Liver (Total Bilb)
0- No Rash 1- Maculopapular rash, <25% of body surface 2- Maculopapular rash, 25-50% of body surface 3- Rash on >50% of body surface, or generalized erythroderma 4- Generalized erythroderma with bullous formation and/or desquamation	0- ≤500 mL/day or <280 mL/m^2/day 1- >500 but ≤1000 mL/day or 280-555 mL/m^2/day 2- >1000 but ≤1500 mL/day or 556-833 mL/m^2/day 3- >1500 mL/day or 833 mL/m^2/day 4- Severe abdominal pain with or without ileus, or stool with frank blood or melena	0- No protracted nausea and vomiting 1- Persistent nausea, vomiting, OR biopsy showing acute GVHD of stomach or duodenum	0- <2.0 mg/dL 1- 2.0-3.0 mg/dL 2- 3.1-6.0 mg/dL 3- 6.1-15 mg/dL 4- >15.0 mg/dL

Overall Clinical Grading of Severity of Acute GVHD

	Skin	Liver	Gut	Upper GI
0	None and	None and	None and	None
I	Stage 1-2 and	None and	None	None
II	Stage 3 and/or	Stage 1 and/or	Stage 1 and/or	Stage 1
III	None-Stage 3 with	Stage 2-3 or	Stage 2-4	N/A
IV	Stage 4 or	Stage 4	N/A	N/A

15.3 Appendix C: Chronic GVHD grading

15.3.1 GVHD scoring criteria stated per Jagasia et al, 2015 (62)

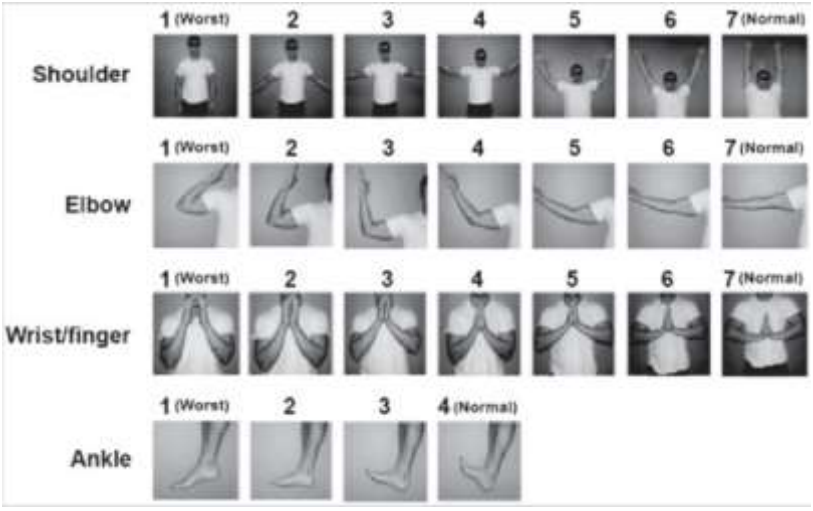
	Score 0	Score 1	Score 2	Score 3
Performance score:_____ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out 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		☐ Superficial 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)*:_____				

† 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.

	Score 0	Score 1	Score 2	Score 3
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):*_____				
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% within 3 months ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss within 3 months (<5%)	☐ Symptoms associated with mild to moderate weight loss within 3 months (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss within 3 months >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 >5x ULN	☐ Elevated total bilirubin >3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

	Score 0	Score 1	Score 2	Score 3
LUNGS **Symptom score:**	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath at after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring O_2)
Lung score: % FEV1 _____ *Pulmonary function tests* ☐ Not performed ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____	☐ FEV1≥80%	☐ FEV1 60-79%	☐ EFV1 40-59%	☐ FEV1 ≤39%
**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.				
JOINTS AND FASCIA P-ROM score (*see below*) Shoulder (1-7):___ Elbow (1-7):___ Wrist/finger (1-7):___ Ankle (1-4)___ ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify)*:_____	☐ 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 decreased 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.)

Photographic Range of Motion (P-ROM)



	Score 0	Score 1	Score 2	Score 3
GENITAL TRACT (see Appendix-XXD‡) ☐ 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)*:_____				
‡ To be completed by specialist or trained medical providers (see Appendix-XXD: GENITAL GVHD AND SCORING FORM)				
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_____		☐ Eosinophilia > 500/µl_____	
☐ Pericardial Effusion_____	☐ Peripheral Neuropathy_____		☐ Platelets <100,000/ µl_____	
☐ Pleural Effusion(s) _____	☐ Polymyositis_____		☐ Others (specify): _____	
☐ Nephrotic syndrome	☐ Weight loss > 5% within 3 months without GI symptoms___			
Overall GVHD Severity (*Opinion of the evaluator*)	☐ **No GVHD**	☐ **Mild**	☐ **Moderate**	☐ **Severe**

Abbreviations: ECOG indicates 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.

15.3.2 Signs and symptoms of chronic GVHD

Sign and symptoms of chronic GVHD per Jagasia et al., 2015 (62)

Organ or site	Diagnostic (Sufficient to Establish the Diagnosis of chronic GVHD)	Distinctive[1] (Seen in chronic GVHD, but Insufficient Alone to Establish a Diagnosis)	Other Features or Unclassified Entities[2]	Common[3] (Seen with Both Acute and chronic GVHD)
Skin	Poikiloderma Lichen planus—like features Sclerotic features Morphea- like features Lichen sclerosus—like features	Depigmentation Papulosquamous lesions	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritus
Nails		Dystrophy Longitudinal ridging, splitting or brittle features Onycholysis Pterygium unguis Nail loss (usually symmetric, affects most nails)		
Scalp and *body hair*		New onset of scarring or nonscarring scalp alopecia (after recovery from chemoradiotherapy) Loss of body hair Scaling	Thinning scalp hair, typically patchy, coarse or dull (not explained by endocrine or other causes) Premature gray hair	
Mouth	Lichen planus-like changes	Xerostomia Mucoceles Mucosal atrophy Ulcers Pseudomembranes		Gingivitis Mucositis Erythema Pain
Eyes		New onset dry, gritty, or painful eyes Cicatricial conjunctivitis KCS Confluent areas of	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	

Organ or site	Diagnostic (Sufficient to Establish the Diagnosis of chronic GVHD)	Distinctive[1] (Seen in chronic GVHD, but Insufficient Alone to Establish a Diagnosis)	Other Features or Unclassified Entities[2]	Common[3] (Seen with Both Acute and chronic GVHD)
		punctate keratopathy		
Genitalia	Lichen planus-like features Lichen sclerosus-like features	Erosions Fissures		
Females	Vaginal scarring or clitoral/labial agglutination	Ulcers		
Males	Phimosis or urethral/meatus scarring or stenosis			

Organ or site	Diagnostic (Sufficient to Establish the Diagnosis of chronic GVHD)	Distinctive[1] (Seen in chronic GVHD, but Insufficient Alone to Establish a Diagnosis)	Other Features or Unclassified Entities[2]	Common[3] (Seen with Both Acute and chronic GVHD)
GI Tract	Esophageal web Strictures or stenosis in the upper to mid third of the esophagus		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss Failure to thrive (infants and children)
Liver				Total bilirubin, alkaline phosphatase > 2 x upper limit of normal ALT > 2 x upper limit of normal
Lung	Bronchiolitis obliterans diagnosed with lung biopsy BOS[4]	Air trapping and bronchiectasis on chest CT	Cryptogenic organizing pneumonia Restrictive lung disease[5]	
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to fasciitis or sclerosis	Myositis or polymyositis[6]	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hyper-gammaglobulinemia Autoantibodies (AIHA, ITP) Raynaud's phenomenon	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality or	

Organ or site	Diagnostic (Sufficient to Establish the Diagnosis of chronic GVHD)	Distinctive[1] (Seen in chronic GVHD, but Insufficient Alone to Establish a Diagnosis)	Other Features or Unclassified Entities[2]	Common[3] (Seen with Both Acute and chronic GVHD)
			cardiomyopathy	

ALT indicates alanine aminotransferase; AIHA, autoimmune hemolytic anemia; ITP, idiopathic thrombocytopenic purpura.

1. In all cases, infection, drug effect, malignancy, or other causes must be excluded.
2. Can be acknowledged as part of the chronic GVHD manifestations if diagnosis is confirmed.
3. Common refers to shared features by both acute and chronic GVHD.
4. BOS can be diagnostic for lung chronic GVHD only if distinctive sign or symptom present in another organ.
5. Pulmonary entities under investigation or unclassified.
6. Diagnosis of chronic GVHD requires biopsy.

15.3.3 NIH global severity of chronic GVHD

Criteria for chronic GVHD per Jagasia et al., 2015 (62)

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:

In skin:

Higher of the 2 scores to be used for calculating global severity.

In lung:

FEV1 is used instead of clinical score for calculating global severity.

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.

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).

15.4 Appendix D: Karnofsky Performance Scale:

Karnofsky Performance Scale [68]	
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.