A Calcineurin Inhibitor-Free GVHD Prevention Regimen After Related Haploidentical Peripheral Blood Stem Cell Transplantation

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Table of contents

1. Study schema

Title	**A calcineurin inhibitor-free GVHD prevention regimen after related haploidentical peripheral blood stem cell transplantation**
Study type	Single institution phase II interventional
Objectives	Primary objective is to estimate the cumulative incidence of grade II-IV acute GVHD by day 100 after related haploidentical peripheral blood stem cell transplantation using the GVHD prophylaxis regimen PTCy, sirolimus and mycophenolate mofetil. Secondary objectives are: - To determine the cumulative incidence of chronic GVHD by 1 year. - To examine additional transplantation outcomes including malignancy relapse, overall survival, progression-free survival, non-relapse mortality, and engraftment and immune reconstitution. - To examine peripheral blood markers of immune tolerance development. - To evaluate the impact of the use of haploidentical related donors in the access of ethnic/race minority patients to allogeneic transplantation.
Sample size	32 evaluable patients (pending statistics)
Study duration	3 years
Patient selection criteria	**Inclusion Criteria:** - Age: Must be older than 18 years, no upper age limit. - Karnofsky performance status $\geq$ 80% for full intensity, $\geq$ 60% for reduced intensity conditioning. - Adequate organ function: LVEF > 45%, FEV1/FVC/aDLCO $\geq$ 50%, AST/ALT<2 times ULN, estimated creatinine clearance $\geq$50 ml/min. - Signed informed consent. - Included diseases: Acute leukemia in first or subsequent CR, CML, primary myelofibrosis, CMML, Intermediate-2 or high risk MDS, NHL in high risk CR1, PIF or beyond CR1 if in PR (SD accepted if no mass>3 cm), HD beyond CR1 if in PR (SD accepted if no mass>3 cm), MM in CR/VGPR.

	Exclusion criteria: - Uncontrolled active bacterial, viral, fungal infection. - Prior alloHCT. - Patient unwilling or unable to comply with study requirements. - Patient with active, progressive or advanced disease based on diagnosis.
Donor selection criteria	In the absence of an allele level matched (8/8 HLA A, B, C and DR) sibling or unrelated donor, all potential related haploidentical donors will be typed. (See algorithm in page 16). A haploidentical donor will share at least one haplotype with the patient. A haploidentical donor will be excluded if the recipient has HLA antibodies against the donor HLA. Among several potential haploidentical donors, in order of priority: Matched CMV IgG serologic status between donor and recipient, ABO matched > minor ABO mismatch > major ABO mismatch, younger donor, male donor.
Treatment plan	**Conditioning regimens:** Myeloablative conditioning: Fludarabine 30 mg/m2 IV daily for 4 days. Dose will be adjusted for estimated creatinine clearance (see SOP BMT-G-115). Busulfan IV dosing targeted for a daily total AUC 5300 mmol*min/L for 4 days.. Busulfan AUC will be pharmacokinetically targeted. AUC 3500 may be used in patients over 60 years of age or with HCT-CI of 2 or more. Chemotherapy may start on day -6 or -5 depending on admission day. Reduced intensity conditioning: Fludarabine: 30 mg/m2 daily on days -6, -5, -4, -3 and -2. Dose will be adjusted for estimated creatinine clearance (see SOP BMT-G-115). Cyclophosphamide 14.5 mg/kg/day on days -6, -5 and total body irradiation 200 cGy on day -1. **Graft:** Donor peripheral blood hematopoietic cells for a target yield of 5 x 10^6 CD34+ cells/kg, minimal accepted number is 2 x 10^6 CD34+ cells/kg recipient IBW, maximum accepted 10 x 10^6 CD34+ cells/kg. **GVHD prophylaxis:** • Cyclophosphamide (50 mg/kg IBW daily dose) will be given on days +3 and +4 post-transplant as an IV infusion over 1-2 hours (see BMT-

	G-119). • Sirolimus (SIR) will be administered as a 9 mg oral loading dose on day +5, followed by maintenance 4 mg daily, target level 8 to 14 ng/ml. In the absence of acute GVHD, sirolimus taper will start on day +90 (+/- 10 days) and it is suggested to finish by day +180. • Mycophenolate mofetil (MMF) will start on day +5 at a dose of 15 mg/kg every 8 hours IV with the maximum daily dose not to exceed 3 gm. MMF will be changed to PO when feasible and discontinued on day +35 (without taper) in the absence of acute GVHD. **Growth factor support:** G-CSF IV or SQ will start on day +5, at 5 mcg/kg/day, until ANC $\geq$ 1,000/mm^3 for 3 consecutive days. **Supportive care:** Will follow established SOP BMT G-132. Given higher fevers and potential risk of cytokine release syndrome (CRS) associated with the use of PBSC grafts, patients will be monitored for CRS and if present will be treated according to published guidelines. (Appendix B)
Study procedures baseline	All clinic assessments, labs, procedures needed to assess eligibility are clinical standard of care evaluation done pre-transplantation. Collection of baseline data Research data collection on baseline patient, disease, remission status, transplantation characteristics and collect baseline immune deficiency panel (IMDFP Tregs)
Statistical considerations	With a total of 32 evaluable patients enrolled, we will have 90% power (α = 0.1) to demonstrate a reduction from 40% to 20% in cumulative incidence of grade II-IV acute GVHD. Approximately 40 patients will be accrued to reach 32 evaluable. Any enrolled, but non-evaluable subject will be replaced in case there was a clinical indication to modify or change the transplant conditioning regimen. The cumulative incidence of acute and chronic GVHD will be estimated, considering malignancy relapse and non-relapse death as competing risk events.

2. Background and rationale

Hematopoietic cell transplantation (HCT) from HLA identical related or unrelated donors or from umbilical cord blood units can cure malignant and non-malignant hematologic, immune and metabolic disorders. Major limitations for wider use of HCT remain timely identification of suitable donors and risk of transplant related morbidity and mortality.

Donor availability and race/ethnic background

The likelihood of finding an optimal donor varies with racial and ethnic background. According to data from the NMDP the highest probability is among whites of European descent at 75%, with a much lower probability in ethnic minorities such as African Americans at 19% or Hispanics at 34%. Optimal cords are only found in a small proportion of patients, 17% for white Europeans, 1% for African Americans and 5% for Hispanics. By using suboptimal donors and suboptimal cord-blood units, a donor may be found in 90% of patients.[1] However, the use of suboptimal donors (mismatched adult or cord-blood units) is associated with a higher rate of complications (graft failure, graft vs. host disease, infection) and lower survival. Patients from ethnic/race minorities more frequently suffer this burden.[2] In addition, once unrelated adult donors are identified, donor attrition decreases the number of donors actually available and willing to donate. Donor attrition is more common in donors from ethnic/race minorities.[3]

We previously performed a comprehensive analysis of unrelated donor searches (n=531) conducted for new HCT consult patients seen in the Moffitt BMT Program (March 2006 to December 2009). Among other major findings, this analysis demonstrated the following: (1) The likelihood of finding a suitable (7/8 or 8/8, i.e. single mismatch or full match at HLA-A, -B, -C, or –DRB1) unrelated donor was significantly dependent on race/ethnicity; all non-Caucasian groups had lower rates of successful donor identification. (2) Among those with a suitable donor, race/ethnicity remained a significant obstacle to actual HCT utilization. The other strongest factor in this domain was disease progression, which speaks to the adverse impact of prolonged wait from donor search to transplant.[4]

These data speak to the need for prompt and successful donor identification for racial/ethnic minorities (hence the rationale for rapid identification of nearly universally available haploidentical donors), and also support the overall feasibility of our planned trial: Non-Caucasian groups made up 26% of our published consecutive series of unrelated donor searches, and current Moffitt BMT Program data suggests that figure has increased to 29%. With our current allogeneic HCT activity (approximately 195 allogeneic HCT procedures done per year, with approximately double that seen in initial consult – expected rate of attrition around 50% with current donor selection practices), 29% of these numbers would result in over 50 minority allogeneic HCT procedures per year (or > 100 minority allogeneic HCT consults). Our proposed study would be highly feasible under these terms, but would become even more feasible if the option of haploidentical transplant was implemented much earlier in the donor search algorithm for minority groups. Importantly, increasing numbers of consults done in Puerto Rico are expected to continue to increase the proportion of minorities in our overall consultation and transplant populations.

Haploidentical related donors

HLA haplotypes (HLA-A, HLA-B, HLA-C, HLA-DR) are tightly linked genes that are jointly inherited. A potential related donor that shares one haplotype with the recipient (4/8 identical), usually a sibling, a parent or a child is available in over 95% of patients. Haploidentical related donors in general, are rapidly identified, readily available and highly motivated to donate. Such donors can be selected at the time siblings are HLA typed and before an unrelated donor search is formally started. Furthermore, haploidentical related donors are available for any patient independent of ethnic/racial background.[5]

Haploidentical Transplantation (HaploT)

The fundamental obstacle of crossing the HLA barrier in HaploT arises from the intense bidirectional responses from T-cells reacting to allogeneic HLA molecules resulting in overwhelmingly high incidence of GVHD and graft rejection.[6] The first effective approach used to overcome these limitations was developed by Aversa et al. with the use of megadoses of CD34+ cells and profound T-cell depletion of the graft. Although effective at decreasing graft failure and facilitating engraftment, this strategy led to slow immune reconstitution and as a

consequence high mortality due to opportunistic infection and relapse.[7] More recently, T-cell replete HaploT emerged as an attractive option owing to the development of stronger and more effective GVHD-preventive strategies namely the use of a short course of cyclophosphamide early after graft infusion.[8,9]

George Santos demonstrated that a short course of high dose cyclophosphamide (PTCy) soon after bone marrow transplantation in rodents targeted and depleted donor and host alloreactive T-cells.[10] This model was subsequently translated to human HCT. The John Hopkins and the Fred Hutchinson Cancer Research Center assessed the use of a non-myeloablative conditioning followed by PTCy (50 mg/kg days +3, +4) and mycophenolate mofetil/tacrolimus prophylaxis after a haploidentical marrow graft infusion in 210 patients with acute leukemia. Engraftment was sustained at 87%; grade 2-4 aGVHD occurred in 27% and cGVHD in 15%. Cumulative incidence of relapse and non-relapse mortality (NRM) were 55% and 18% respectively. This low NRM was counterbalanced by a significant relapse rate in this high risk population.[11] This experience has been duplicated by others and has compared favorably with historical data using HLA-identical sibling and unrelated donors.[12-16] Importantly, higher rates of GVHD have been seen when peripheral blood stem cells are used for transplantation (an overall observation that recapitulates extensive evidence in the setting of matched sibling and matched unrelated donor transplantation).[17-20]

A Treg-favoring GVHD prophylaxis

Our group has previously shown that a sirolimus (SIR) based GVHD prophylaxis leads to significantly less grade 2-4 acute GVHD compared to the standard of care methotrexate/tacrolimus in adult recipients of HLA identical sibling and unrelated donors. The main benefit was in gastrointestinal GVHD. As acute GVHD remains a significant cause of early transplant associated morbidity and mortality, effective acute GVHD prevention is an important clinical goal. Furthermore, SIR-based GVHD prophylaxis also led to a lower incidence of moderate-severe chronic GVHD. Because chronic GVHD remains the main cause of late morbidity and mortality after HCT, this represents an advance on HCT. In addition, sirolimus treated patients had a significantly greater proportion of regulatory T cells (Treg) among the CD4+ cells in the peripheral blood, and isolated Treg were functional.[21,22]

Calcineurin-inhibitor (CNI) based GVHD prophylaxis has been commonly used as GVHD prevention strategy on HaploT. However, CNI negatively affect regulatory T-cells which appear to be critical to establish tolerance after HCT, in particular if using peripheral blood stem cell grafts. In contrast, SIR fosters post-transplantation Treg recovery which has been shown to be central in tolerance development after transplantation. Besides its Treg promoting ability and its pleiotropic immunosuppressive properties, SIR has been shown to have an antiviral and antitumor activity.[23]

Therefore, SIR-based GVHD prophylaxis is a desirable approach after HaploT to further improve acute and chronic GVHD prevention and to facilitate tolerance. Recently, Cieri et al. published a clinical study on 40 patients using a sirolimus based approach, a myeloablative conditioning and peripheral blood stem cell grafts followed by PTCy. In their hands, it was a safe and effective approach, with a rapid recovery of T cell compartment and in particular of Tregs. In particular, the cumulative incidence of grade II-IV acute GVHD in this trial was only 15%, while prior trials (using predominantly tacrolimus and MMF on the backbone of PTCy) have rather reported ≥ 40% grade II-IV acute GVHD when using peripheral blood as graft source. This major difference in outcome forms the key rationale for our testing this regimen, and provides an estimated effect size for powering our trial.[24]

We want to further expand this experience using both, myeloablative and reduced intensity conditioning and peripheral blood stem cell grafts to allow our patients to have a safe and comprehensive approach that would be applicable within a large range of age and comorbidities. In addition, this approach would mitigate the impact of race and ethnicity in the availability of suitable donors and would allow more patients to access HCT.

Tolerance associated gene-expression after HCT

Clinical transplantation tolerance is broadly defined as the absence of ongoing immunologic injury due to donor-recipient incompatibility without ongoing immunosuppressive therapy (IS). Clinical judgment does not accurately identify the development of immune tolerance after HCT, and therefore IS taper and discontinuation is empiric and frequently complicated by GVHD. In previous investigation, Pidala, and colleagues examined peripheral blood transcriptional markers in tolerant and non-tolerant HCT recipients with the goals of developing an accurate phenotypic

classifier and dissecting biologic mechanisms of immune tolerance following HCT. The total study sample included 15 HCT recipients who demonstrated sustained immune tolerance, 17 HCT recipients with established chronic GVHD on immune suppression, and 10 healthy control subjects. Comprehensive data on patient characteristics, prior and current GVHD activity and IS therapy were collected. Peripheral blood mononuclear cells (PBMC) were isolated from a single time point freshly obtained peripheral blood sample to (1) characterize immune cell populations by surface phenotype, and (2) extract RNA for microarray analysis using the Affymetrix Human U133 plus 2.0 array. Time from HCT to sample collection did not differ between the tolerant and non-tolerant groups (median 38.5 vs. 39.5 months, p =0.97). There were no significant differences between groups in total CD4+ T cells, total CD8+ T cells, αα or αβ CD8+ cells, memory or effector CD8+, regulatory T cells (CD4+CD25+CD127low), total CD14+ monocytes, total CD19+ B cells, plamacytoid or monocytoid dendritic cells, NK, or NKT cells. Significance analysis of microarray (SAM) was used to identify genes differentially expressed between phenotypic groups. A final tolerance gene set included 281 probe sets that distinguished the tolerant group from both non-tolerant and controls. A final non-tolerant gene set included 122 probe sets that distinguished non-tolerant patients from both the tolerant group and healthy controls. Functional Ontology Enrichment (MetaCore by GeneGo) identified enriched process networks including NK cell cytotoxicity, antigen presentation, lymphocyte proliferation, and cell cycle and apoptosis. An accurate classifier (>90% accuracy, correctly classifying 14/15 tolerant cases and 15/17 non-tolerant cases) was developed only utilizing 20 probe sets. Differential expression of highly discriminative genes was confirmed using NanoString nCounter technology. In total, these data demonstrate that differential gene expression in PBMC can provide insight into the biology of immune tolerance after HCT, and can be used to develop a classifier with high degree of accuracy to identify tolerant patients.[25] We now propose to use this technology to prospectively investigate tolerance development following PTCy/SIR/MMF therapy in the setting of HLA-haploidentical peripheral blood stem cell transplantation.

Summary rationale:

Racial/ethnic minorities suffer disparate access to transplant, largely driven by failure to identify suitable adult unrelated donors. This represents a particular shortcoming of our current practices at Moffitt, given the diversity of our usual patient population seen for BMT consultation. This

innovative trial will directly address this need through efficient identification of haploidentical donors for such patients. While this overall work-flow enhances access to HCT, it also makes original investigation possible to optimize outcome of haploidentical transplantation. We will examine a novel GVHD prevention strategy to prevent morbidity and death associated with GVHD, and expand current scientific knowledge in this area through correlative science focused on the immunobiology of GVHD and transplantation tolerance.

3. Trial objectives

a. Hypothesis:

A calcineurin inhibitor-free GVHD prevention regimen with the addition of sirolimus to post-transplant cyclophosphamide and mycophenolate mofetil will allow adequate prevention of acute GVHD in the setting of haploidentical peripheral blood hematopoietic cell transplant.

b. Primary objective:

To estimate the cumulative incidence and severity of acute GVHD by day 100 after related haploidentical peripheral blood stem cell transplantation using the GVHD prophylaxis regimen PTCy, sirolimus and mycophenolate mofetil (MMF).

c. Secondary objectives:

1. To determine the cumulative incidence and severity of chronic GVHD by 1 year.

2. To examine additional transplantation outcomes including malignancy relapse, overall survival, progression-free survival, non-relapse mortality, and engraftment and immune reconstitution.

3. To examine peripheral blood markers of immune tolerance development.

4. To evaluate the impact of the use of haploidentical related donors in the access of ethnic/race minority patients to allogeneic transplantation.

4. Trial endpoints

a. Primary endpoint:

Cumulative incidence of grade II-IV acute GVHD by day 100 after HCT. Acute GVHD organ staging and assessment of overall grade will use standard consensus criteria.[26]

b. Secondary endpoints:

1. Cumulative incidence and severity of chronic GVHD by 1 year.

    - Chronic GVHD diagnosis and severity scoring will follow NIH Consensus guidelines.[27]

2. Evaluate following outcomes at 100 days and 1 year:

    - Overall survival defined as time from transplant to death or last follow-up.

    - Progression-free survival defined as the minimum time interval from transplant to relapse/recurrence, to death or to last follow-up.

    - Cumulative incidence of non-relapse mortality.

    - Cumulative incidence of relapse/progression: Defined as hematologic relapse or any unplanned intervention to prevent progression of disease in patients with evidence (molecular, cytogenetic, flow cytometric, radiographic) of malignant disease after transplantation.

3. Rate of infectious complications: Data on all infections grade III and IV according to standard criteria (CTCAE v 4.0) will be collected.

4. Time to hematopoietic recovery (ANC and platelets) defined as the first of 3 consecutive days with ANC above 500 cells/uL and the first of 3 consecutive days

with a platelets count of 20,000/uL or higher (without transfusions in preceding 7 days).

5. Donor chimerism (CD3, CD33, unsorted BM) at day 30, 90, 180, and 365.
6. Immune reconstitution
    1. Will be collected at baseline, day 30, 90, 180 and 365.
    2. This will be assessed through collection of a routinely available clinical laboratory immune deficiency panel.
7. Serial research blood sample collection for tolerance biomarker studies will be collected at day 21, 90, and day 180.
8. Detailed race/ethnicity data will be collected on patients getting haploidentical donors or unrelated donors.

5. Patient population

a. Patient <u>inclusion</u> criteria

1. Age: Patient must be older than 18 years. There will be no upper limit of age. Comorbidities and vital organ function will define eligibility criteria.
2. Karnofsky performance status: Full intensity conditioning, 80-100%; reduced intensity conditioning, 60-100%.
3. Vital organ function:
    - Cardiac: Left ventricular ejection fraction must be > 45% assessed by MUGA scan or echocardiogram. No myocardial infarction within 6 months of transplant evaluation.
    - Pulmonary: FEV1, FVC, and adjusted DLCO must be $\geq$ 50% of predicted values.

- Liver: Transaminases (AST, ALT) < 2 times upper limit of normal values.

- Kidney: Estimated creatinine clearance ≥ 50 cc/min.

4. Signed informed consent.

5. Included disease conditions and remission status

    a. Acute leukemia in CR1 or second/subsequent CR.

    b. Chronic myeloid leukemia, primary myelofibrosis, chronic myelomonocytic leukemia..

    c. Int-2 or high risk MDS

    d. Hodgkin lymphoma beyond CR1 with chemosensitive disease, SD may be included if no mass>3 cm.

    e. Non-Hodgkin lymphoma in high risk CR1 or subsequent CR (by clinical, cytogenetic or molecular criteria), PIF or relapsed with chemosensitive disease. SD may be included if no mass>3 cm.

    f. Multiple myeloma in CR/VGPR.

6. Patient will be <u>excluded</u> if there is:

    a. Active bacterial, viral, fungal infection not controlled with appropriate antimicrobial therapy.

    b. Prior allogeneic HCT.

    c. Patients unwilling to comply with study requirements.

    d. Patients with active, progressive or advanced disease based on diagnosis.

b. Donor selection:

1. Per MCC BMT program practices, an allele level matched (8/8 HLA A, B, C and DR) sibling or unrelated donor is preferred. If a matched donor is not found, mismatched unrelated or haploidentical donors may be considered. See suggested algorithm for donor selection on page 17.

2. If a haploidentical donor is considered, parents, children, full siblings and in selected cases, extended family, will have high resolution typing at the MCC HLA laboratory. A familiar haploidentical donor is chosen among those who share at least one HLA-A, B, C, DRB1 and DQB1 haplotype with the patient.

3. Patient will be screened for antibodies targeting mismatched HLA antigens in potential haploidentical donors (donor specific antibodies, DSA). Antibody screen and confirmatory testing using Luminex single-antigen-bead test will be done.

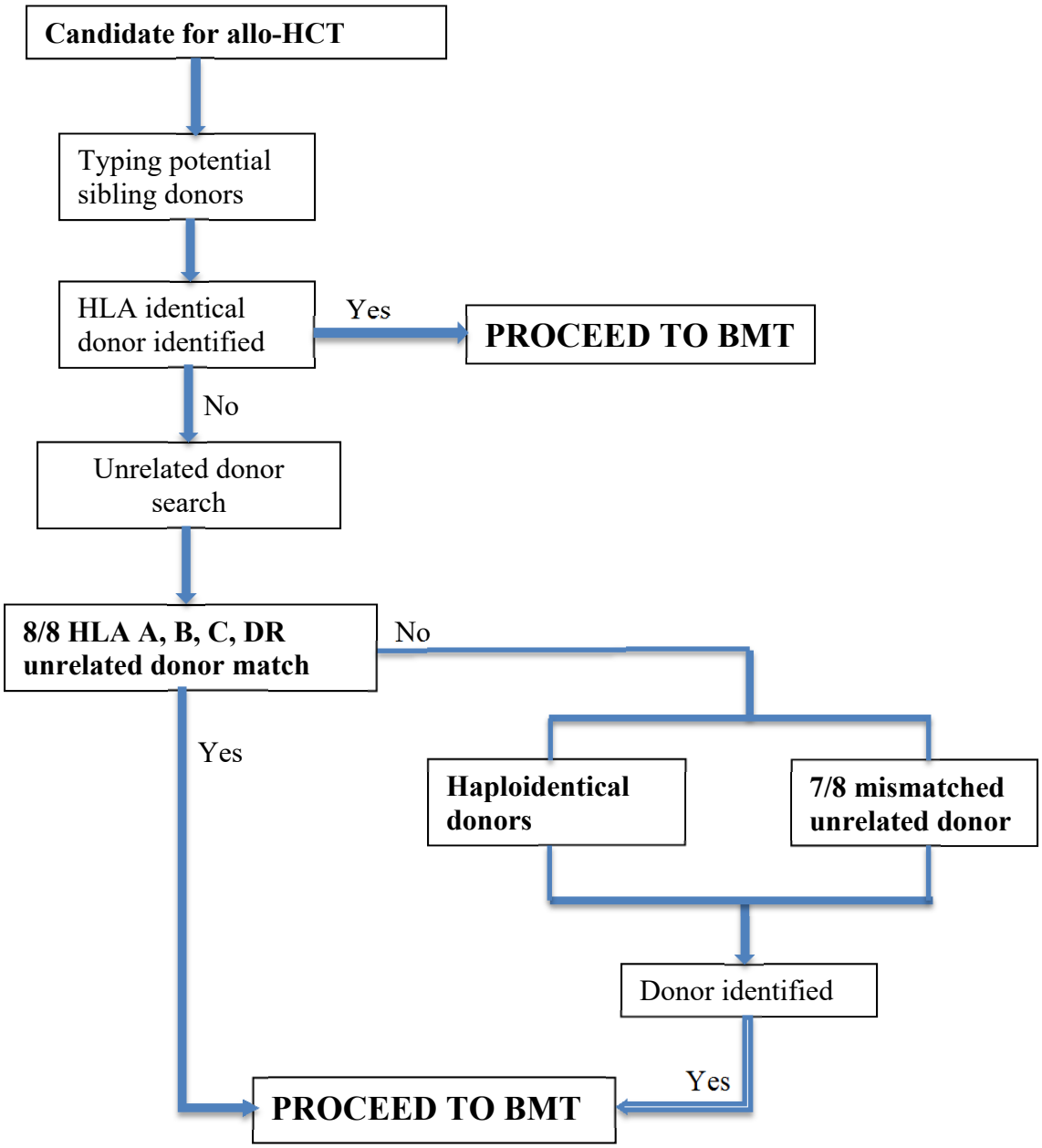
4. Among several potential donors, will choose in order of priority:

    a. Matched CMV IgG serologic status between donor and recipient.

    b. ABO-matched donor preferred, then minor ABO mismatch, then major ABO mismatch.

    c. Younger donor preferred: child, then sibling, and then parent

    d. For male recipient, male donor will be preferred. Avoid mother as a donor unless no other choices.



Donor selection for allogeneic HCT

6. Study design and treatment Plan

This will be a single arm, prospective, single institution interventional trial.

a. Conditioning regimen

Several transplant conditioning regimens are routinely employed in standard practice. To reduce heterogeneity, two commonly used myeloablative (MAC) and reduced intensity (RIC) regimens are permitted on this trial. The intensity of the conditioning regimen will be decided by primary investigator based on performance status, vital organ function and comorbidities:

1. Myeloablative conditioning:

   - Fludarabine: 40 mg/m^2 daily for 4 days. Dose will be adjusted for estimated creatinine clearance (see SOP BMT-G-115).

   - Busulfan: IV dosing targeted for a daily total AUC 5300 mmol*min/L for 4 days. Busulfan AUC will be pharmacokinetically targeted (see SOP BMT-G-111). An AUC 3500 mmol*min/l may be considered in patients over 60 years of age or with multiple comorbidities. Chemotherapy may start on day -6 or day -5 depending on the day of admission (-6 for Wednesday admission, -5 for Sunday admission).

2. Reduced intensity conditioning:

   - Fludarabine: 30 mg/m^2 daily on days -6, -5, -4, -3 and -2. Dose will be adjusted for estimated creatinine clearance (see SOP BMT-G-115).

   - Cyclophosphamide 14.5 mg/kg/day on days -6, -5.

   - Total body irradiation 200 cGy on day -1.

b. Peripheral Blood Hematopoietic Cell Transplantation

On day 0, patients will receive a peripheral blood hematopoietic cell graft. Donor peripheral blood hematopoietic cells will be harvested for a target yield of 5 x 10^6 CD34+ cells/kg, maximum accepted number is 10 x 10^6 CD34+ cells/kg, minimal accepted number is 2 x 10^6 CD34+ cells/kg recipient IBW. Total amount of T-cells in the graft will be noted.

c. GVHD prophylaxis

1. Cyclophosphamide 50 mg/kg IBW daily dose will be given on days +3 and +4 post-transplant as an IV infusion over 1-2 hours. Hydration will start 2 hours prior and Mesna will be given in divided doses IV, pre- and post-cyclophosphamide according to MCC guidelines (BMT-G-119).

2. Sirolimus (SIR) will be administered as a 9 mg oral loading dose on day +5, followed by maintenance. SIR levels will be monitored and maintenance dosing adjusted as needed for a target trough level 8 to 14 ng/ml, per Moffitt BMT program standard practice (BMT SOP #103). In the absence of acute GVHD, sirolimus taper will start on day +90 (+/- 10 days) and it is suggested to finish by day +180.

3. Mycophenolate mofetil (MMF) will start on day +5 at a dose of 15 mg/kg every 8 hours IV with the maximum daily dose not to exceed 3 gm. MMF will be changed to PO and discontinued on day +35 (without taper) in the absence of acute GVHD.

d. Growth factor support

G-CSF will be given beginning on day 5 at a dose of 5 mcg/kg/day (rounding to the nearest vial dose), until absolute granulocyte count (ANC) is $\geq$ 1,000/mm^3 for three consecutive days. G-CSF may be given IV or subcutaneously.

e. Supportive Care

Supportive care, including management of Non-infectious fever, transfusion, infection prevention, and nutritional support will be managed according to established SOP BMT G-132. As the proportion of T-cells in a peripheral blood graft may be 5 to 10 times higher than in bone marrow, there is evidence suggesting that higher fever and possibly cytokine release syndrome (CRS) may happen in these patients shortly (hours to a few days) after graft infusion.[29] Patients will be monitored for CRS and may be treated with tocilizumab according to published guidelines. (Please see appendix B).

7. Data collection and safety monitoring

Procedures on this trial will follow the Data & Safety Monitoring Plan (DSMP) adopted by the H. Lee Moffitt Cancer Center & Research Institute. This ensures that all clinical research conducted or coordinated by the Cancer Center is scientifically well designed, responsibly managed, appropriately reported and protects the rights and welfare of human participants. The methods and amount of monitoring required are dictated by the degree of risk involved to the individual participants and the complexity of the clinical research. Other entities that will assume responsibility for data and safety monitoring include: Principal Investigators (PI); Scientific Review Committee (SRC); Protocol Monitoring Committee (PMC); Research Compliance Division (RCD) of the Cancer Center's Corporate Compliance Office; Institutional Review Board (IRB). Full details of this DSMP are provided in Appendix A.

8. Patient enrollment and evaluation

a. Baseline evaluation

The baseline study visit encompasses the following:
Eligibility determination (review inclusion and exclusion criteria).

a. All clinic assessments, labs, procedures needed to assess eligibility are clinical standard of care evaluation done pre-transplantation.

b. Collection of baseline data

i. Research data collection on baseline patient, disease, remission status, transplantation characteristics.

c. Collect baseline immune deficiency panel (IMDFP Tregs).

b. Post-transplant evaluations

Post-transplantation study visits occur on day 30, 90, 180, and at 1 year (with acceptable windows as outlined in the study calendar).

All clinic assessments and laboratory studies needed to inform study endpoints captured at these time points are part of clinical standard of care (with exception of underlined research samples).

1. Day 21 study visit

- collect day 21 research blood sample

2. Day 30 study visit

- Capture ANC/PLT engraftment data, day 30 disease response assessment and donor chimerism, and any occurrence of acute GVHD
- monitor AE/SAE throughout observation period
- Collect day 30 immune deficiency panel IMDFP Tregs.

3. Day 90 study visit

- Capture acute GVHD data, day 90 disease response assessment and donor chimerism, and death events.
- Capture initiation of sirolimus taper.

- monitor AE/SAE throughout observation period
- Collect day 90 immune deficiency panel IMDFP Tregs
- collect day 90 research blood sample

4. Day 180 study visit

- Capture acute and chronic GVHD data, day 180 disease response assessment and donor chimerism, mortality events
- Capture time of sirolimus discontinuation
- monitor AE/SAE throughout observation period
- Collect day 180 immune deficiency panel IMDFP Tregs
- collect day 180 research blood sample

5. 1 year study visit

- capture chronic GVHD data, 1 year response assessment and donor chimerism, mortality events
- monitor AE/SAE throughout observation period
- Collect 1 year immune deficiency panel IMDFP Tregs

c- Relapse

Relapsed patients will be considered off-trial and will be followed only for date of death.

Table 8.1 Summary of patient clinical assessment

Study assessments	Baseline	21 days (+/-3)	30 days (+/-7)	90days (+/-10)	180 days (+/-14)	365 days (+/-21)
Baseline pre-transplant assessment: - History, physical exam, weight, Karnofsky performance status, infectious disease markers, EKG, LVEF, pulmonary function tests - Disease staging - Pregnancy test	X					
Neutrophil and platelet engraftment			X			
Donor chimerism			X	X	X	X
Acute GVHD			X	X	X	X
Chronic GVHD				X	X	X
Disease response			X	X	X	X
Death			X	X	X	X
AE/SAE	X		X	X	X	X
IDP-Treg	X		X	X	X	X
Research blood sample		X		X	X	

Notes:

9. Statistical considerations

The primary objective of this single-arm phase II trial is to estimate the cumulative incidence of grade II-IV acute GVHD by day 100 after transplantation. The historical benchmark for grade II-IV acute GVHD in this setting (related haploidentical transplantation, peripheral blood stem cell graft, conventional immune suppression prophylaxis including PTCy, tacrolimus and MMF) is ≥ 40%. The preliminary data from Cieri and colleagues suggests that a novel approach (above treatment program, but use of sirolimus and MMF) results in only 15% grade II-IV acute GVHD by day 100 post-HCT. With a total of 32 evaluable patients enrolled, we will have > 90% power ($\alpha = 0.1$) to demonstrate a reduction from 40% to 20% in our trial. With an anticipated accrual of 1-2 patients per month, our planned accrual timeline is consistent with the overall grant period. Approximately 40 patients will be accrued to reach 32 evaluable. Any enrolled, but non-evaluable subject will be replaced in case there was a clinical indication to modify or change the transplant conditioning regimen.

The cumulative incidence of acute and chronic GVHD will be estimated, considering malignancy relapse and non-relapse death as competing risk events. Overall and progression-free survival will be estimated using the Kaplan-Meier method. Stratified survival curves will be compared using the log-rank test. Patient characteristics and other factors of interest will be associated with overall and progression-free survival using Cox proportional hazard model. Rates of other complications will be estimated with allied confidence interval.

10. Tolerance biomarker assessment

Sample collection: Research blood samples will be drawn at day 21, day 90, and day 180 after HCT. These **time points** are highly feasible, and chosen for the following reasons: (1) The day

90 sample will serve as the primary single time point predictor for subsequent complete IS discontinuation, as we expect most patients would have stopped MMF by that point, and would be in the midst of SIR taper to discontinuation. The day 21 sample permits an earlier single time point assessment to determine whether findings at day 90 are present at earlier post-HCT time points. (2) The inclusion of both day 21 and day 90 samples permits a secondary approach of examining change in gene expression from day 21 to 90 as a predictor of subsequent tolerant vs. not tolerant phenotype; we expect this will be highly informative of evolution of tolerance mechanisms over time. (3) These single time point samples are also positioned for prediction of GVHD syndromes as major clinical indicator of alloreactivity. Day 21 samples will predate the majority of acute GVHD events, and day 90 is ideal for predictive analyses for development of chronic GVHD. Finally, the day 180 sample should capture the majority of subjects at time of immune suppression discontinuation. This sample will be used as predictor at time of immune suppression stop for subsequent sustained tolerance vs. development of GVHD after immune suppression discontinuation. **Sample characteristics:** 10mL peripheral blood will be drawn at each time point. Based on expected yield of 0.5-1ug RNA/mL whole blood, total RNA isolated will be more than sufficient for planned mRNA profiling. RNA will be isolated (total RNA, inclusive of small RNA to permit future microRNA studies), and RNA integrity confirmed.

Clinical phenotypes: The tolerant phenotype of interest in the primary analysis (inclusive of both single time-point prediction and longitudinal change) is immune tolerance as defined by complete discontinuation of IS and absence of GVHD by the end of study follow up period. In contrast, non-tolerant is defined by either/both continued IS or GVHD. As secondary objectives day 21 and day 90 samples will be examined for prediction of alloreactivity (acute and chronic

GVHD, respectively). Definitions of acute and chronic GVHD will follow standard consensus criteria.[26,27]

C1.3. Methodology and Analyses

Identify transcriptional markers predictive of immune tolerance development

We have performed power calculations based on a two-sided two-sample t-test (PASS software) to estimate the required sample size for the initial discovery cohort. We examined power according to the proportion of differentially expressed probe sets at an effect size of 2 with a false discovery rate of 10%. With n=15 vs. n=15 in the respective phenotypic groups, we have > 90% power to detect differential expression of as low as 1% of the total probe sets tested. As our prior SAM two-group comparison (*see preliminary data*) showed n=655 (~1% of total probes) differentially expressed, n=30 will be used for discovery.

We will identify differentially expressed genes that distinguish tolerant vs. non-tolerant patients using microarray (Affymetrix Human U133 plus 2.0 array). Scanned output files will be analyzed by robust multi-array average analysis (RMA).[30] The Significance Analysis of Microarrays (SAM) technique will be employed to identify differentially expressed genes between groups,[31] using 10% FDR, and ≥ 1.5 fold difference in mean expression values. Functional Ontology Enrichment (MetaCore by GeneGo) with 5% FDR filter will be used to identify enriched pathways and cellular process networks. We will confirm candidate markers identified using NanoString nCounter technology.

Investigate longitudinal change in transcriptional markers for prediction of immune tolerance development

Approach: We will employ linear models for microarray data with a FDR of 10% for this analysis examining change in gene expression (day 21 to day 90) to subsequent tolerant vs. non-tolerant phenotype.[32,33] As a secondary approach, we will also use generalized linear mixed effect modeling to identify genes with longitudinally different change between groups. A predictive model based on genes identified above will be developed using the machine learning technique, including the PCA method and support vector machine. The final predictive model will take into account both the identified gene signature, and also significant clinical variables.

Examine predictive markers of acute and chronic GVHD

Approach: We will study day 21 and day 90 samples for prediction of acute and chronic GVHD, respectively. This will provide key information for the following: (1) Differentially expressed genes predictive of GVHD will be compared to those segregating the ultimate tolerant and non-tolerant phenotype to address the extent to which the tolerant transcriptional program reflects absence of GVHD-associated determinants vs. induction of a specific tolerance-inducing program. Shared and unique genes among these two gene lists will be determined, mapped to biologic pathways, and compared to prior data. (2) GVHD predictive biomarkers published to date have not been studied in this clinical context (HLA-disparate haploidentical transplants

using PTCy/SIR/MMF). We anticipate unique findings in this setting that will contribute to mechanistic understanding of failure/GVHD development in this setting.

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Appendix A

Data Safety and Collection Monitoring

a. Data collection

The Principal Investigator and assigned clinical research staff (clinical research coordinator, research data specialist) will be responsible for maintaining all study related documents. The Moffitt electronic medical record (PowerChart) will serve as source document. Data collected will be stored in Moffitt Cancer Center's database system, ONCORE. Identifying patient information will be kept confidential. Representatives of the USF IRB will have access to patient information as it pertains to the study. Privacy and confidentiality of the information will be protected to the extent provided by law.

b. Safety monitoring

The principal investigator will have the primary responsibility for data safety and monitoring. Input will be sought from sub-investigators and other members of the BMT Program concerning data and safety issues. The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the SRC and IRB. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to a DSMB and/or to the PMC and IRB as required, that adverse events are reported according to protocol guidelines, and that any adverse actions reflecting patient safety concerns are appropriately reported.

The investigators and members of the BMT Research Staff will meet regularly. The following data will be reviewed:

- Rate of accrual
- Adverse events
- Protocol deviations and/or violations.

- If necessary, corrective action and/or educational programs will occur to ensure subject safety and data integrity. Reports to the SRC, PMC, and IRB will be submitted as required.

c. Anticipated toxicity following allogeneic transplantation:

There are numerous anticipated adverse consequences of transplantation, which include, but are not limited to conditioning regimen related toxicity such as severe mucositis, idiopathic pneumonia syndrome, hepatic veno-occlusive disease and death, early and late infectious complications, potentially severe or fatal acute or chronic graft vs. host disease, and relapse of primary disease and its complications. Thus, expected complications of transplantation will not be collected as adverse events on this trial.

d. Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

e. Adverse event collection and reporting

Adverse Event

An adverse event is any unexpected medical occurrence associated with the use of a drug or therapy in humans, whether or not considered drug related. It can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease, temporally associated with the use of a drug. Infection, acute and chronic GVHD will not be considered adverse events.

AE Grading Criteria:

AE severity is graded on a scale from 1 to 5 according to NCI CTCAE v4.03 or current version. Adverse events not included in the NCI CTCAE should be recorded and graded according to the General Grade Definition provided below:

ADVERSE EVENT GENERAL GRADE DEFINITIONS

Grade 1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate	Minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL*
Grade 3	Severe	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**
Grade 4	Life-threatening	Life-threatening consequences; urgent intervention indicated
Grade 5	Death related to AE	Death related to AE

*Instrumental ADL: Preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL: Bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Suspected Adverse events – a subset of Adverse Events based on causality

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the event. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the event.

Unexpected

An adverse event is considered unexpected if it is not listed in the investigator brochure, occurs in severity greater than previously described, or - for the purpose of this protocol - if it is not in keeping with expected post-transplantation toxicity.

Serious

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

1. Death
2. A life-threatening adverse event (places the subject at an immediate risk of death)
3. Inpatient hospitalization or prolongation of existing hospitalization
4. A persistent or significant disability or incapacitation
5. A congenital anomaly or birth defect

Additionally, events that jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes above may also be considered serious based on appropriate medical judgment.

Adverse event recording:

Non-serious AE: Only unexpected, grade 3-5 AE will be recorded.

Serious AE: All SAE will be recorded.

Adverse event reporting:

Non-serious AE: Unexpected, grade 3-5 AE will be reported to the IRB in summary form on an annual basis.

SAE: The principal investigator will report each serious adverse event, regardless attribution, within 24 hours of learning of the occurrence. In the event that the principal investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the principal investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. The SAE report must include event term(s), serious criteria, and the investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration.

Appendix B

Management of Cytokine Release Syndrome (CRS) after Haploidentical transplantation

CRS is a non-antigen specific toxicity that occurs as a result of a high level immune activation. This immune activation is associated with modern cellular immunotherapies and is usually required as a mediator for clinical benefit.

It manifests when large numbers of lymphocytes and/or myeloid cells become activated and release inflammatory cytokines. It has been classically associated with the infusion of monoclonal antibodies like anti-CD3 (OKT3), anti-CD52 (alemtuzumab), anti-CD20 (rituximab) among others. It has also been reported with the infusion of bi-specific antibodies for leukemia (blinatumomab), haploidentical cells to patients with refractory leukemia, adoptive immunotherapies for cancer and most notably following the infusion of T-cells engineered to express chimeric antigen receptors (CAR T-cells) to treat leukemia or lymphoma.

Symptoms usually begin shortly after cell infusion (within 1 to a few hours) and coincide with peak of cytokine release. Multiple cytokines are elevated, initially TNFα, and then IFNγ, IL-1β, IL-2, IL-6, IL-8 and IL-10. Commonly, in severe CRS, patients will develop progressive organ dysfunction and will require aggressive support in intensive care unit. Emerging evidence has implicated IL-6 as a central mediator of toxicity in CRS as the initiator of the pro-inflammatory cascade. C-reactive protein (CRP) is an acute phase reactant produced in the liver largely in response to IL-6, and CRP level serve as a reliable surrogate of IL-6 activity. During CRS, it increases by several logs. Rapid increase of CRP has been seen in patients at risk for severe CRS.

In haploidentical transplantation, patients receive a donor hematopoietic cell graft containing large numbers of T-cells. Upon antigen presentation, donor and host alloreactive T-cells are activated and enter a rapid proliferation phase. This phase is heralded by the development of high grade fevers in day +1 to +3 after graft infusion. Fever rapidly abates after administration of cyclophosphamide in days +4, +5.

The bulk of the published data using marrow grafts suggests that this febrile period is rarely associated with organ dysfunction. In this protocol, we will use peripheral blood stem cell grafts, which in average, have 1 more log of T-cells compared to marrow. It is likely that this large increase in T-cells may generate a stronger T-cell activation/expansion and proportionally stronger cytokine release with a potential major clinical impact. Very little data is available in the literature on how to address this concern. As a safety measure we will incorporate an algorithm for the management of CRS that follows the guidelines published by Lee et al.,[29] and has been used successfully in other settings, particularly after infusion of CAR-T cells. Table 1 summarizes the clinical manifestations of CRS and table 2 has the algorithm for management.

Table 1. Clinical manifestations of CRS.

Organ system	**Symptoms**
Constitutional	Fever ± rigors, malaise, fatigue, anorexia, myalgias, arthralgias, nausea, HA
Skin	Rash
Gastrointestinal	Nausea, vomiting, diarrhea
Respiratory	Tachypnea, hypoxemia
Cardiovascular	Tachycardia, hypotension, ↑ cardiac output, ↓ cardiac output (late)
Renal	Azotemia
Hepatic	Transaminitis, hyperbilirubinemia
Neurologic	Headache, AMS, confusion, delirium, hallucinations, tremor, seizures
Coagulation	Elevated D-dimer, hypofibrinogenemia

Table 2. Algorithm for the management of CRS

Cytokine Release Syndrome Grading assessment	Extensive co-morbidities or older age? No/Yes	Treatment
Grade 1: • Fever (defined as ≥ 38.3°C) • Constitutional symptoms	N/A	• Vigilant supportive care • Assess for infection • Treat fever and neutropenia if present, monitor fluid balance, antipyretics, analgesics as needed
Grade 2: • Hypotension: responds to fluids or one low dose vasopressor • Hypoxia: responds to <40% O^2 • Organ toxicity: grade 2	No	• As above for grade 1 • Monitor cardiac and other organ function closely
Grade 2: • Hypotension: responds to fluids or one low dose vasopressor • Hypoxia: responds to <40% O^2 • Organ toxicity: grade 2	Yes	• As above for grade 2 • Consider tocilizumab
Grade 3: • Hypotension: requires multiple vasopressors or high dose vasopressors • Hypoxia: requires ≥ 40% O^2 • Organ toxicity: grade 3, grade 4, transaminitis	N/A	
Grade 4 • Mechanical ventilation • Organ toxicity: grade 4 excluding transaminitis	N/A	

Tocilizumab dose will be 4 mg/kg and may be repeated if clinical improvement does not occur on 24 to 48 hours. The goal of the treatment of CRS in haploidentical transplantation is the prevention of organ dysfunction, while preserving the GVHD prevention effect associated with the use of post-transplantation cyclophosphamide. In addition to the clinical evaluation during the first 3 days after graft infusion, it is recommended to follow CRP on a daily basis until day +5. Rapid increases in CRP should be taken in consideration in conjunction with the clinical information to diagnose and trigger the treatment of CRS.