Abbreviated Title: HCT for T-cell disorders

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Title: Phase II Trial of Allogeneic Hematopoietic Cell Transplantation for Disorders of T-cell Proliferation and/or Dysregulation

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Commercial Agents: Equine anti-thymocyte globulin (Atgam), Pentostatin, Cyclophosphamide, Tacrolimus, Mycophenolate Mofetil, Busulfan

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PRÉCIS

Background:

- Disorders of T-cell proliferation and/or dysregulation (TCP/D) can lead to T-cell lymphoproliferative disorders, autoimmunity, infection, and aberrant immune activation with resulting organ dysfunction, morbidity, and mortality.
- Allogeneic hematopoietic cell transplantation (HCT) has the potential to cure disorders of TCP/D.
- Subjects with TCP/D may be at higher risk for graft rejection and/or disease relapse.

Primary Objective:

- Separately by arm: To estimate the percentage of recipients with >50% donor T cell chimerism *and* graft-failure free survival at day +180 post-HCT

Eligibility:

- Age ≥ 4 years
- TCP/D deemed to be of sufficient past severity to warrant HCT that meets at least one of the criteria below:
    - Identified germline T-cell activating mutation in the PI3k pathway
    - Identified ADA2 deficiency (biallelic mutations in CECR1 (ADA2) and/or phenotypically with low ADA2 level) leading to neutropenia requiring chronic GCSF therapy or to transfusion-dependent anemia or thrombocytopenia
    - T-cell infiltration of liver, spleen, lymph nodes, marrow, lungs, gut, or other organs by T cells, as evidenced by laboratory, radiographic, and/or anatomic pathology evaluation, resulting in organ dysfunction and/or organomegaly
    - Latent herpesvirus infection in T lymphocytes
    - History of or active evidence of hemophagocytic lymphohistiocytosis (HLH)
    - Recurrent or prolonged fevers attributed to immune dysregulation
    - T-cell population in blood and/or marrow with immunophenotype of large granular lymphocytes (LGL), with or without clonality or lymphocytosis
    - T-cell lymphoproliferative disorder in the setting of an underlying immune defect
    - Immune-mediated cytopenias of one lineage requiring transfusion or GCSF support or of 2 or 3 lineages with or without transfusion or support
    - Chronic active Epstein-Barr virus (EBV)
- At least one potentially suitable 7-8/8 HLA-matched related or unrelated donor, or an HLA-haploidentical related donor
- Adequate end-organ function
- Not pregnant or breastfeeding
- HIV negative
- Disease status: Subjects with malignancy should be referred in remission for evaluation if possible, although the aggressive nature of many of these diseases necessitates the potential need to enroll subjects onto study and treat with standard therapies before proceeding to protocol therapy (HCT)

Design:

- There will be two arms that vary in conditioning intensity – an immunosuppression-only conditioning (IOC) arm for high-risk subjects and a reduced-intensity conditioning (RIC) arm.
- IOC arm: equine anti-thymocyte globulin (e-ATG) 40 mg/kg/day IV on days -14 and -13, pentostatin 4 mg/m^2/day IV on days -9 and -5, low-dose cyclophosphamide orally daily on days -9 through -2
- RIC arm: e-ATG 40 mg/kg/day IV on days -14 and -13, pentostatin 4 mg/m^2/day IV on days -11 and -7, low-dose cyclophosphamide orally daily on days -11 through -4; busulfan IV, pharmacokinetically dosed, on days -3 and -2.
    - Subjects will be assigned to the IOC arm if there is significant end-organ dysfunction present and it is felt that a conditioning regimen that includes busulfan would likely be associated with intolerable or life-threatening toxicities for the subject. Subjects will also be assigned to the IOC arm if they possess a DNA repair defect, telomere maintenance defect, or familial cancer predisposition syndrome that necessitates limiting chemotherapy as much as possible to prevent future cancer risk.
- Peripheral blood stem cells are the preferred graft source, although bone marrow is permitted
- GVHD prophylaxis:
    - PTCy on days +3 and +4 (50 mg/kg/day on RIC arm and 25 mg/kg/day on the IOC arm, with the option of 25 mg/kg/day on the RIC arm), tacrolimus on days +5 through +90, and MMF on days +5 through +25.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Separately by arm: To estimate the percentage of recipients with >50% donor T cell chimerism *and* graft-failure free survival at day +180 post-HCT

1.1.2 Secondary Objectives

- By arm, to estimate the cumulative incidences of acute graft-versus-host disease (aGVHD) at 1- year post-HCT, chronic GVHD (cGVHD) at 1 and 2 years post-HCT, primary graft failure at day +60 post-HCT, secondary graft failure at 1- year post-HCT, and transplant-related mortality (TRM) at day +180 and 1- year post-HCT
- By arm, to determine the kinetics and durability of engraftment and lineage-specific donor chimerism, including:
    - The association between early chimerism data (days +21, +28, +35, and +42) and primary or secondary graft failure at day +60 post-HCT
    - The percentage of donor T-, B-, NK-, and myeloid cell populations at days +28, +42, +60, +100, +180, and 1 year after HCT
- By arm, to estimate the probabilities of event-free survival (EFS), GVHD-free graft failure-free survival (GGFS), GVHD-free relapse-free survival (GRFS), and overall survival (OS) at 1, 3, and 5 years post-HCT
- By arm, to estimate the cumulative incidence of Epstein-Barr virus (EBV), cytomegalovirus (CMV), JC virus (JCV), BK virus (BKV), adenovirus, and human herpesvirus 6 (HHV6) detection in blood at day +100 post-HCT

1.1.3 Exploratory Objectives

- To determine whether HCT reverses the clinical phenotype of TCP/D at 1- year post-HCT. Disease-specific studies of immune system phenotype and function will also be evaluated at 1 year for comparison to the pre-HCT state. The relationship between reversal of phenotype and engraftment of donor cell subsets will be evaluated at 1 year
- To determine the activity of e-ATG against T-cell and non-T cell lineages in blood and marrow
- To determine the frequency of virus-associated reactivation, viral infection, viral disease, and viral lymphoproliferation after HCT, including serial assessment of EBV, CMV, HHV6, BK virus, adenovirus, and JC virus through 1- year post-HCT
- To evaluate the incidence of other post-HCT infections in the 1- year post-HCT, including complications, treatment, duration, and control
- To determine the incidence of invasive fungal infections at day +180 post-HCT
- To evaluate the dynamics of immune reconstitution and changes in cytokine/soluble marker profiles after HCT, including changes related to the development of viral reactivation, severe infection, virus-associated malignancy, and GVHD
- To evaluate the need for and response to subsequent donor cell infusions after HCT
- To evaluate the development of virus-specific immunity after HCT
- To further elucidate the degree of donor chimerism necessary to reverse the disease phenotype in various T-cell-mediated disorders
- To evaluate the impact of HCT on endocrine function at 1- year post-HCT
- To evaluate changes in cytokine profiles during conditioning and post-HCT and correlate with clinical events
- To compare immune reconstitution, chimerism kinetics, and clinical endpoints between recipients of IOC conditioning and RIC conditioning
- To identify and describe the incidence and clinical course of complications, sequelae, and outcomes of HCT in this heterogeneous and unique population, on an individual, case series, arm, or study level, as appropriate and relevant.

1.2 BACKGROUND AND RATIONALE

1.2.1 Introduction

Disorders of TCP/D are a heterogeneous group of diseases wherein aberrant T cell proliferation, survival, and/or function instigate the disease pathology. As a result, disorders of TCP/D can be associated with significant morbidity and mortality. HCT offers a potentially curative therapy for patients with disorders of TCP/D.(1)

Over 200 different immunodeficiency diseases have now been phenotypically and genetically described and this number will inevitably grow with the wider use of sophisticated genetic testing.(2) Many of these immunodeficiency diseases can phenotypically manifest as disorders of TCP/D.(2) However, not all patients who have a pathogenic mutation in one of these genes will have the same phenotype and even patients with the same mutation of the same gene may have different phenotype and severity of disease. Thus, identification of patients with TCP/D will not depend solely on their mutation (if one is identified) but will also be heavily informed by their disease phenotype.

As an example, some patients with CECR1 mutations leading to deficiency of adenosine deasminase 2 (DADA2) have T-cell mediated immune cytopenias as the sole manifestation of their disease.(3) Others with DADA2 have an autoinflammatory phenotype.(4) The specific mutations in CECR1 do not seem to predict phenotype. While the autoinflammatory phenotype is best described and was central to the initial descriptions of this disease, the T-cell mediated immune phenotype is now being recognized and is an area of active, ongoing study between Dr. Daniel Kastner's group within the National Human Genome Research Institute of NIH, in conjunction with Dr. Dimitrova (Office of Training and Education) and Dr. Jen Kanakry within the Center for Immuno-Oncology (CIO) (formerly Experimental Transplantation and Immunology Branch (ETIB)) of NCI, Dr. Neal Young's group within the National Heart, Lung, and Blood Institute, and Dr. Kathy Calvo of the Hematology Section of the Department of Laboratory Medicine.

Another group of patients, those with activating PI3k mutations or other mutations that drive activity of or inhibit suppression of the PI3k/Akt pathway, in general have been shown to be difficult to engraft, in the limited experience throughout the world to date.(5) It has been the consensus among us and others with experience transplanting patients with PI3k mutations that these patients are difficult to engraft. On our existing protocol 16-C-0003, we have transplanted 3 patients using the RIC approach and have had one primary graft failure and one patient who, although at present (2 years out from HCT) remains phenotypically reversed and without any of her prior disease manifestations, has mixed donor chimerism. The third patient has excellent donor chimerism. Other transplanters have transplanted a handful of patients with PI3k patients and have turned to myeloablative conditioning given difficulties with primary and secondary graft failure. While myeloablative conditioning may be an answer for these hard to engraft diseases, many PI3k patients, by nature of their underlying disease, have poor organ function and would not be candidates for myeloablative conditioning. Furthermore, others who might be candidates for myeloablative conditioning may not be best served by receiving these high doses of conditioning if less intensive approaches could be equally effective at securing engraftment, as there are both short and long-term toxicities associated with myeloablative conditioning. This response by others in the field to intensify conditioning in hard to engraft diseases by using myeloablative approaches is a very standard approach to minimizing the risk of graft failure. However, myeloablative conditioning is unlikely to be the answer that leads to progress and advancement in the field of HCT for these diseases, given its associated short- and long-term toxicities, its prohibitive levels of chemotherapy for patients with significant organ dysfunction, and the impossibility of autologous hematopoietic recovery after graft failure. For these reasons, activating mutations in the PI3k pathway will be one of the criteria that would make a patient eligible for HCT on this protocol, over HCT on the currently open 16-C-0003 trial described above.

On 16-C-0003, we have successfully transplanted one patient with CTLA4 haploinsufficiency, on the RIC-MMF arm, using a peripheral blood stem cell (PBSC) graft and a matched sibling donor. The PBSC graft and matched sibling donor may have favored engraftment in what would be expected to be a difficult to engraft disease. The patient also received abatacept and sirolimus right up until his conditioning started, which may have improved his inflammatory milieu peri-HCT to help with engraftment. In a case series of 8 HCTs for CTLA4 haploinsufficiency by Slatter et al, where the genetic diagnosis was retrospective in 7 of 8 recipients, all received RIC matched donor T-cell replete grafts (5 PBSC, 3 marrow) with conventional GVHD prophylaxis

and there were no graft failures and 2 transplant-related mortalities.(6) These patients were all pre-treated prior to transplant with immunosuppressive therapy. While end-organ damage, such as insulin-dependent diabetes, was not reversed, patients did see improvement and/or resolution in their enteropathies, immune cytopenias, and immune dysregulation. The rates of GVHD, however, were high, occurring in 50% of patients. Thus, although RIC, serotherapy-containing regimens appear promising in these patients, improvements can still be made to reduce toxicities of HCT and improve overall outcomes and immune function. In diseases such as these where there are alternative, targeted therapies, determining how to best transplant those who require it requires ongoing investigation, ideally suited for study at the NIH.

Success in HCT for non-malignant TCP/D disorders relies solely on prompt engraftment of a new, functional immune system. Thus, the goal of HCT for disorders of TCP/D is to ensure successful engraftment of affected cell lineages while minimizing transplant-associated toxicities, including GVHD, infection, and the potentially damaging effects of chemotherapy.

1.2.2 Improving outcomes of HCT for disorders of TCP/D

There are several key areas to address when striving to improve HCT outcomes for patients with TCP/D disorders. These include safety and toxicity, feasibility and donor availability, donor selection, timing, optimized pre-HCT disease control, and minimization of infectious complications peri-HCT, all of which will be discussed below.

Minimizing Conditioning Intensity: HCT conditioning regimens can vary in intensity from fully myeloablative to those that are purely immunodepleting. Conditioning regimens containing high doses of alkylating agents such as busulfan (> 8 mg/kg) or total body irradiation of 8 Gy fractionated or higher, are considered myeloablative conditioning (MAC), meaning that autologous hematologic recovery will not occur in the event of graft failure.(7) MAC may be associated with lower relapse rates in some hematologic malignancies, but is also associated with increased TRM and transplant-related toxicities.(8, 9) These toxicities include sinusoidal obstructive syndrome (SOS), infertility, growth retardation, organ dysfunction, and risk of secondary malignancies. MAC may also carry a higher risk of GVHD due to increased gastrointestinal mucosal disruption and associated antigen presentation. Co-morbidities present prior to HCT also factor into decisions regarding conditioning intensity, as the presence of multiple co-morbidities are associated with inferior outcomes and certain co-morbidities may pose particularly increased risks for poor outcomes if MAC is used. Furthermore, many patients may be unable to tolerate intensive conditioning regimens as a result of active infection, end-organ damage, or underlying issues with DNA repair/telomere maintenance. There is a growing body of literature to support the use of non-myeloablative (NMA)/RIC in HCT for non-malignant diseases and a shift in the HCT field in general towards immunoablation rather than myeloablation.(10-13) These NMA/RIC approaches that rely on immunodepletion rather than myeloablation are typically less toxic and associated with lower TRM, as well as fewer long-term complications. There is growing recognition that adequate immunoablation alone is required for successful allografting. However, engraftment can be more difficult with less intense conditioning and mixed chimerism or graft failure are more often seen in the RIC setting.(14, 15) Apart from myeloid malignancies, where myeloablative conditioning has been shown to be of benefit for PFS and a trend towards improved OS in a randomized, phase III study, (16) myeloablative conditioning has a vanishing role in the transplantation of lymphoid and non-malignant diseases. Optimizing RIC approaches while still minimizing the toxicities that come

with intensified therapies, is important. Clinical research related to the application of novel RIC HCT approaches to patients with diseases that are either historically difficult to engraft and/or achieve disease control and cure could improve outcomes and the applicability of HCT to diseases that, at present, have low success rates with HCT. RIC HCT that can achieve effective immunoablation without myeloablation should translate into improved engraftment and lower TRM, providing better disease control and higher cure rates in these difficult disorders of TCP/D.

Expanding HCT Availability Through Use of Alternative Donors: While the use of alternative donor sources is essential to expand the availability of HCT to any patient who requires the procedure, the toxicities associated with greater human leukocyte antigen (HLA) disparity or minor histocompatibility antigen mismatch, namely graft failure and GVHD, have historically been significant barriers in the field. The availability of matched unrelated donors (MUDs) through donor registries has increased donor options for patients requiring HCT, but continues to leave many patients, particularly racial minorities, without fully-matched donor options. The odds of finding a MUD for patients of some ethnic minorities can be less than 10%. Furthermore, potential MUDs are not always available at the time a transplant is needed and the costs associated with procuring a MUD are also a consideration. Nonetheless, contemporary outcomes of MUD HCT are largely comparable to outcomes reported with matched related donor (MRD) HCT and thus the use of MUDs is accepted to be safe and reasonable when a MRD is not available.(17-19)

Umbilical cord blood (UCB) is another alternative donor source, but is associated with delayed engraftment, poor immune reconstitution, high rates of EBV-associated posttransplantation lymphoproliferative disorder (EBV-PTLD), and unfavorable rates of GVHD, rendering this alternative donor option less desirable in the setting of HCT for hard to engraft diseases and disorders of the immune system.(20) In addition, second donor collections, for donor lymphocyte infusion or additional hematopoietic stem cells is not possible with UCB.

Modern approaches to HCT using HLA-haploidentical (haplo) donors has greatly changed the field with regard to providing a readily available donor option to virtually any patient in need of transplantation. Historically, the use of haplo donors has been associated with increased risk of GVHD as well as graft rejection due to HLA disparity between donor and recipient.(21) While T-cell depletion of the graft is one approach to reduce the GVHD associated with haplo HCT, the associated risk of graft failure, EBV-PTLD, and delayed immune reconstitution make this approach particularly unfavorable for patients with difficult to engraft diseases. By contrast, Johns Hopkins University has developed an approach to facilitate haplo HCT using PTCy that is associated with high rates of engraftment, low rates of GVHD, and low TRM that were superior to those seen with UCB grafts upon comparison of parallel phase II studies.(22-24) PTCy is an approach to GVHD prophylaxis that works *in vivo* to immunomodulate donor T cells, although the underlying mechanisms are still under active study, predominantly by Dr. Christopher Kanakry of the Center for Immuno-Oncology (CIO) (formerly Experimental Transplantation and Immunology Branch (ETIB)) within the National Cancer Institute (NCI).(25, 26) Multiple transplant centers across the world have adopted the PTCy platform for haplo HCT, reproducing and confirming Hopkins' favorable results, with low rates of TRM, graft failure, grade 3-4 aGVHD, and cGVHD.(27-32) At present, there is clinical equipoise in using haplo donors when MRDs are not readily available, as outcomes associated with haplo HCT using PTCy are

equivalent to outcomes with MUDs and even MRDs.(28) Advantages of using a haplo donor include immediate donor availability, access to donor-derived cellular therapies if needed after HCT, and the ability to select a donor from several available relatives based on non-HLA factors. Despite this major advance in the field, there is more limited and less successful experience using PTCy for the transplantation of patients with hard to engraft diseases and these approaches need to be further optimized.(23, 33-36)

Haplo Donors: The current study will use MRD, MUD, and haplo donors, prioritizing young (age < 40 years) MRD over alternative donor sources. In cases where there is no young MRD option, a MUD or haplo related donor will be used, with the choice between these two donor sources left to the discretion of the PI. Fewer than 25% of patients will have a MRD who is healthy and medically appropriate to donate. While the lack of a MRD should not discourage the use of HCT in TCP/D patients who need the procedure, there remains some reluctance in the field regarding the use of alternative donors - particularly for non-malignant diseases - given that there can be associated higher rates of GVHD. As a result, MRDs have remained the much preferred donor source for patients undergoing HCT, posing significant limitations when a MRD is not an option. Among alternative donor sources, MUDs have been used most commonly for HCT when MRDs are not available.(37-39) Apart from the more extensive experience with the use of haplo grafts in severe combined immunodeficiencies (SCID),(40) there remains at present a relative lack of global experience with haplo HCT for disorders of TCP/D. T-cell depleted haplo grafts, while highly effective in preventing GVHD, have been unsuccessful in immune system disorders due to the high risk of graft rejection, as well as infectious complications. However, recent advances in approaches to alternative donor HCT, notably including those that use T-cell replete grafts, have been associated with outcomes that compare quite favorably to those following MRD HCT.(41, 42) However, despite well-established successes with T-replete haplo HCT using PTCy platforms, the HLA barrier does still present an additional hurdle to successful engraftment, translating into more difficulty engrafting specific diseases with this approach. Based on personal experience as well as colleagues' anecdotal experiences, T-cell replete haplo HCT for severe disorders of TCP/D such as hemophagocytic lymphohistiocytosis or chronic active EBV (CAEBV) is associated with high rates of graft failure. Thus, the use of alternative donors in these disorders of TCP/D represents an area that warrants active clinical investigation to further advance the field. In the present study, we plan to draw from not only MRD donor sources when available, but also use MUD or haplo related donors for patients who do not have a MRD.

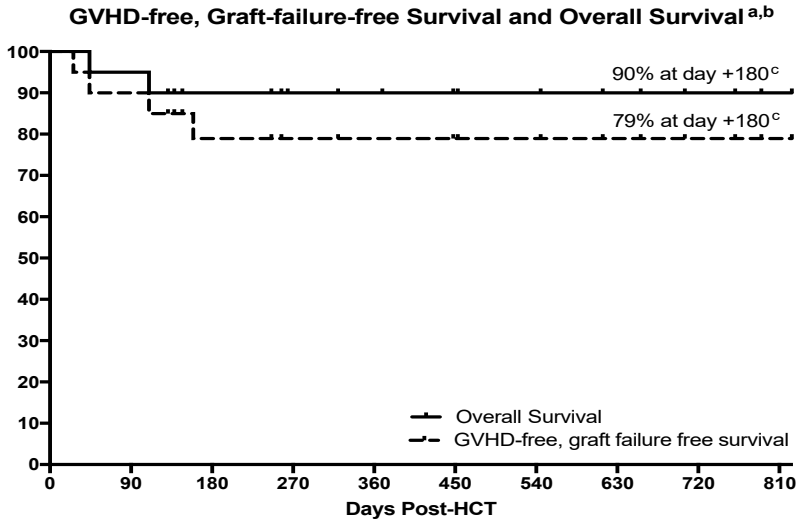


Figure 1. Graft-failure-free, GVHD-free survival and overall survival for the RIC arm of 16-C-0003.

Results of the 16-C-0003 RIC Arm: Considering the advantages of PTCy, including low toxicities, rapid immune reconstitution, low rates of infectious complications,(28, 43, 44) a PTCy-based HCT platform has been studied within the NCI for the transplantation of patients with primary immunodeficiency diseases (16-C-0003, NCT02579967) since it opened in October, 2015. In this trial, the RIC arm has fully accrued (n=20 HCT recipients), with at least 180 days of post-HCT follow-up of survivors. For the RIC arm's primary endpoint, the estimated day +180 grade 3-4 aGVHD-free, graft-failure-free survival was 79%, which was very promising and exceeded the study's pre-defined threshold of success of 70%. The RIC arm's 1-year OS estimate was 90%, Figure 1. In addition, the cumulative incidence of 1-year TRM was 10%, which translates into a TRM rate well below what would be expected given the high-risk patients included on the arm. On the 16-C-0003 RIC arm, the median HCT-Comorbidity Index (HCT-CI) was 2.5. HCT-CI scores of 1-2 have been associated in the RIC HCT setting with 2-year estimates of the cumulative incidence of NRM of 29%, with scores of 3 or higher associated with 2-year NRM of 41%.(45) Patients with high-risk diseases, akin to those we expect to enroll on the this trial, have been shown to be more likely to have high HCT-CI scores.(46) Overall, the organ toxicity seen in the RIC arm of 16-C-0003 has been as expected for a regimen that includes busulfan, with low rates of SOS (seen in 1 of 20 RIC arm patients, in a patient with significant underlying nodular regenerative hyperplasia). The potential liver toxicity in patients with TCP/D disorders is of particular importance, as many patients with disorders of TCP/D can have liver infiltration of lymphocytes, hemophagocytosis, inflammatory damage to the liver, or other insults to the liver.

Potential Limitations of the 16-C-0003 RIC Arm Among Specific Disease Phenotypes: While the cumulative incidence of primary or secondary graft failure at day +180 on the RIC arm was 10%, which is well within what would be expected for a RIC approach and may even be better than expected given the use of haplo donors and a conditioning regimen without radiation or serotherapy, we did find that specific patients on the 16-C-0003 RIC arm appeared harder to engraft than others. One patient with primary graft failure had immune cytopenias (thrombocytopenia, neutropenia) unresponsive to rituximab, steroids, and sirolimus as his indication for HCT. Although it was difficult to demonstrate that his immune cytopenias were a T-cell mediated process (T cells were of normal phenotype, without clonal TCR gene rearrangements, although an abnormal TCR rearrangement pattern was detected on pre-HCT evaluation and marrow CD4:CD8 ratio was inverted), his lack of response to rituximab was supportive of a T-cell mediated immune process. As primary graft rejection is a T-cell mediated process (and, by definition, an attack on donor hematopoietic elements), we attribute the patient's graft failure to insufficient host immunosuppression during HCT conditioning, likely as a result of an underlying advantage of host T cells. There was also one patient who had secondary graft failure on the RIC arm after good engraftment of donor myeloid cells. Her pre-HCT marrow showed an inverted CD4:CD8 ratio, although normal T cell immunophenotype and number, with an abnormal TCR gene rearrangement pattern but no abnormal, clonal lymphocyte population. Her very low donor CD3 chimerism early post-HCT was an indication that she had not been sufficiently immunodepleted prior to HCT and what followed, despite donor lymphocyte infusion, was secondary graft failure with complete loss of myeloid chimerism. In addition, on the new RIC-MMF arm of 16-C-0003, we have had 1 patient have secondary graft failure, which occurred in a patient with pre-HCT T-cell mediated immune cytopenias (neutropenia) due to underlying adenosine deaminase 2 deficiency (CECR1 mutation). Post-

HCT, the patient had slow primary engraftment, followed by an abrupt rebound of autologous CD8 cells of LGL phenotype that exerted a quick and severe host vs graft effect on donor cells, resulting in complete secondary graft failure. While the overall results of the accrued RIC arm and currently accruing RIC-MMF arm of 16-C-0003 are promising, these three patients with graft failure stand out among the very heterogeneous group of patients transplanted on 16-C-0003, indicating that while we have seen good results with many diseases, the approach to host immunodepletion may need to be optimized for patients with particularly resilient T cells that can exert a host versus graft attack on donor cells post-HCT.

Limitations of the IOC Arm on 16-C-0003: On 16-C-0003, there is also an IOC arm that aims to avoid all myelotoxic preparative chemotherapy, focusing solely on immunodepletion. This arm has both a high-risk and standard-risk cohort. The high-risk cohort is for patients where a higher level of conditioning would be offered if possible, but underlying organ dysfunction makes more intensified therapy not possible. The standard-risk cohort is for patients who have what is felt to be a sufficiently depleted immune system entering into HCT that only a low level of conditioning is necessary for engraftment. We have enrolled only a few patients on the IOC arm, but there has been secondary graft failure in both patients enrolled on the standard-risk arm. On the high-risk arm, two of the three transplanted patients were evaluable for engraftment and both achieved engraftment, although both remain split chimeras to date. Thus, it is clear that this level of conditioning is too low to achieve engraftment in the majority of patients and needs optimization. At the same time, we recognize that many of our referred patients have significant organ dysfunction and will not tolerate chemotherapy that is myelotoxic, even at the RIC level. Based on these data from 16-C-0003, as well as the projected referrals of patients with disorders of TCP/D, it seems necessary to have a HCT option in this protocol that uses low-levels of conditioning for patients who cannot tolerate more myelotoxic therapy, but that also further increases the immunosuppression beyond what was used in the 16-C-0003 IOC arm.

Safety and Toxicity: The morbidity and TRM of HCT for TCP/D should be low so that the procedure can reasonably be performed before a life-threatening manifestation of the disease occurs. HCT may be the only potentially curative option for these patients and thus minimizing the risks of HCT is critical to improve survival for these patients. Major factors that contribute to transplant-associated toxicities and mortality include 1) graft failure, 2) infection, 3) GVHD, and 4) organ damage from conditioning, including SOS, infertility, cardiopulmonary dysfunction, and endocrine dysfunction, among others. In this study, we have considered the weight of each of these contributing factors for patients with disorders of TCP/D in designing the HCT platform, adjusting aspects of the 16-C-0003 conditioning regimen, graft source, GVHD prophylaxis. With a focus on diseases that have shown to be difficult to engraft on 16-C-0003, the goal of this protocol is to build off the results of 16-C-0003 and further optimize engraftment and pre-HCT disease control while minimizing toxicities, particularly infection and organ dysfunction in these TCP/D disorders.

Donor Selection: In using alternative donors such as MUD or haplo related donors for disorders of TCP/D, a patient may have several donor options available. Selecting the best donor becomes an important aspect of improving outcomes for TCP/D patients. In certain circumstances, the necessity to proceed quickly to HCT or the desire to ensure repeated donor availability may lead to the decision to select a haplo related donor over a MUD. Furthermore, multiple donor options

renders feasible the selection of a donor based on non-HLA factors, including CMV serostatus, EBV serostatus, and donor age.

Timing: A major barrier to improving outcomes for patients with disorders of TCP/D is the fact that many simply arrive at HCT too late - after significant complications have already occurred or multiple unsuccessful lines of chemotherapy, which have immediate as well as lasting effects on overall health and survival. With the development of safer and less toxic approaches to HCT, the potential benefits of earlier HCT for these disorders of TCP/D that are potentially curable with HCT could come to largely outweigh the associated risks, justifying the optimal time for the HCT of many disorders of TCP/D to a timepoint prior to the development of severe organ dysfunction, or life-threatening infection. In the present study, we aim to further study a modified approach to the HCT platform studied in 16-C-0003, with changes that are hypothesized to improve engraftment and disease control while maintaining the favorable rates of GVHD and TRM seen in the prior study, within a population of patients deemed to have difficult to engraft diseases. As some potential transplant candidates may have aggressive diseases that relapse quickly or require ongoing therapy to control as HCT is being arranged, there may be a need to treat patients per standard of care here at the NIH as they are being evaluated for HCT on this protocol. Patients would only be treated at NIH with standard of care therapies if this was a necessary intervention to bridge to HCT on protocol.

1.2.3 Addition of e-ATG to the Immuno-reducing Backbone for Conditioning

Immuno-reducing Backbone: NMA/RIC results in successful engraftment when adequate immunodepletion and immunosuppression of the host immune system is achieved. There are many options in designing NMA/RIC regimens for HCT, the majority of which are fludarabine-based. However, pentostatin – another purine nucleoside analogue – has also been used, albeit less commonly, in HCT conditioning with good tolerability and low TRM.(47) In 16-C-0003, pentostatin and low dose cyclophosphamide (PC) is used as the immuno-reducing backbone of conditioning. This immuno-reducing backbone was designed based on work by Dr. Dan Fowler in the NCI's CIO (formerly ETIB) that demonstrated that pentostatin and fludarabine are equally effective in synergistically depleting host $CD4^+$ and $CD8^+$ T cells when combined with cyclophosphamide.(48) However, pentostatin was more immunosuppressive than fludarabine, as shown by decreased ability of host T cells to secrete interleukin-2 and interferon-γ *in vitro*, decreased host-versus-graft reactivity *in vivo*, and superior engraftment in a murine allograft model with PC as compared to fludarabine plus low-dose cyclophosphamide.(48) The PC regimen effectively reduces the number and function of T-cells within eight days of starting the conditioning therapy.

In a study by Dr. Steven Pavletic (ID-CTP), a NMA, pentostatin-based conditioning regimen was associated with significant immunosuppression of T-, B-, and NK- cells, rapid establishment of donor chimerism across cell lineages in the majority of patients, and minimal non-hematologic toxicities.(49) Based on these data, Pavletic and colleagues concluded that optimal immunosuppression can be achieved as early as seven days after starting pentostatin.(49) An 8-day immune-reducing backbone of PC has been used as the framework to transplant patients on 16-C-0003, with varying doses of busulfan based on conditioning arm intensity. To date, 32 recipients on 16-C-0003 have received this conditioning PC backbone, at either the RIC level (n=26: 20 on the RIC arm, 5 on the RIC-MMF arm, and 1 on an extra slot/emergency extension of the RIC arm) or IOC level (n=6). Tolerability has been excellent, with only one high-risk IOC

arm patient, with significant pre-HCT chronic renal disease and prior liver transplant, having worsened renal insufficiency after the first dose of pentostatin. As a crude measure of lymphodepletion, an absolute lymphocyte count (ALC) of < 100 K/µL on day 0 was targeted. The median ALC on day 0 for the RIC arm was 230 K/µL (range 20-970). The patients on the RIC arm with primary and secondary graft failure had ALCs on day 0 of 240 K/µL and 70 K/µL, respectively. The patient on the RIC-MMF arm with secondary graft failure had such a low WBC count on day 0 that the ALC was not reported. Thus, the degree of lymphodepletion, as crudely estimated by ALC in peripheral blood, has been very variable across patients, and ALC on day 0 may not be the best reflection of total body lymphodepletion nor the best estimate of effective conditioning against graft failure. While the PC backbone was designed for 16-C-0003 to be lymphodepleting, it is clearly not fully lymphodepleting and thus better characterized as immuno-reducing. In diseases that are difficult to engraft, or in malignancies of T cell origin, more intensified approaches to T cell depletion with conditioning may afford benefits to engraftment, as well as disease control in the setting of T-cell malignancy.

Choice of Serotherapy for Additional Immunosuppression: Other approaches to immunoablation for HCT include the use of the anti-CD52 antibody alemtuzumab and ATG.(50, 51) However, rabbit ATG (r-ATG) and alemtuzumab have half-lives of 29 days and 14-21 weeks, respectively.(52, 53) As a result, r-ATG and alemtuzumab, when used in HCT conditioning, both function to condition the host and partially (or fully) *in vivo* T cell deplete the graft. Thus, they are often used as part of the GVHD prophylaxis strategy, particularly for alternative donor HCT,(51),(54) in addition to serving a role in conditioning.(50) When ATG or alemtuzumab are timed during conditioning in such a way that they also T cell deplete the graft, these approaches are associated with increased risk of infectious complications post-HCT, including viral reactivation and disease, fungal infection, poor immune reconstitution, and EBV-PTLD.(55, 56) In registry studies of NHL patients undergoing RIC HCT, proximal ATG use during conditioning, given for GVHD prophylaxis, has been associated with higher risk of TRM on multivariable analysis.(51) More distal administration of r-ATG has been associated with more favorable immune reconstitution post-HCT, but even distal administration of r-ATG likely has an effect on the graft.(57) Horse/equine (e-ATG) has a shorter half-life than r-ATG, with a half-life of 5.7 days.(53) However, because across almost all reported HCT experience, these serotherapies are timed in such a way as to serve the dual function of host immunodepletion and *in vivo* T cell depletion of the graft for GVHD prophylaxis, there is extremely limited experience in using these serotherapies solely for host lymphodepletion. When coupled with a highly effective approach to GVHD prophylaxis, such as PTCy, which immunomodulates but does not deplete the T cells given on transplant day, serotherapy that is timed to *solely condition the host* might result in improved engraftment, as well as favorable immune reconstitution, fewer infectious complications, and low rates of GVHD, resulting in favorable TRM. As e-ATG has the shortest half-life, this is the most appealing agent to use when picking an approach that minimizes the duration of conditioning while still allowing for the serotherapy to wear off before administration of the graft. Thus, e-ATG will be used as part of the conditioning regimen in this protocol, timed so that two half-lives have passed before the graft is given.

E-ATG is most commonly used to treat T-cell mediated immune disorders such as severe aplastic anemia (SAA) and pure red cell aplasia.(58, 59) When compared to r-ATG or alemtuzumab for the treatment of SAA, e-ATG was associated with lower elevations and durations of EBV DNA detection in the blood.(59) Of note, e-ATG has been widely used in

pediatric populations, in the setting of HCT and aplastic anemia and thus its addition to the conditioning used in this protocol is not felt to represent any specific considerations for the pediatric population.

1.2.4 Use of pentostatin in pediatric patient populations

Pentostatin has been used in pediatric patients at the doses similar to those proposed in this study with good tolerability and low toxicity for prolymphocytic leukemia,(60) aGVHD,(61) cGVHD,(62-64) refractory immune cytopenias,(65) and refractory Langerhans cell histiocytosis.(66) A phase I study of pentostatin for aGVHD treatment included children as young as six months of age and found the recommended phase II starting dose of pentostatin to be 1.5 mg/m^2/day for three consecutive days.(67) When pentostatin was used in a randomized, phase II trial of aGVHD therapies, pentostatin had the fewest grade 3-5 toxicities as compared to the three other treatment arms of MMF, denileukin diftitox, or etanercept.(61) In a phase I study of pentostatin for pediatric patients with acute lymphoblastic leukemia, doses ranged from 0.25-1.0 mg/kg IV for three consecutive days. Based on toxicities observed in this trial, a starting dose of 0.5 mg/kg/day was recommended for Phase II studies of pentostatin in pediatric patients.(68) Higher and/or more intensive dose schedules of pentostatin in pediatric patients with T-cell acute lymphoblastic leukemia have been associated with unacceptably high frequency and severity of renal, neuromuscular, and hepatic toxicities.(69) In this protocol, two doses of 4 mg/m^2 separated by three days is less than 0.5 mg/kg/day for three consecutive days, if the typical body surface area for a child is considered to be between 0.5-1.33 m^2, depending on age. Pentostatin has been used in 16-C-0003 in the conditioning of all patients transplanted to date, of which 10 have been pediatric patients. No issues have been seen with pentostatin across all patients and thus no pediatric-specific concerns have arisen.

1.2.5 Rationale for two arms

In the current protocol, e-ATG-PC will be used as the immunodepleting backbone in both conditioning arms. Patients on the IOC arm will receive e-ATG-PC only whereas patients on the RIC arm will receive e-ATG-PC and 2 days of pharmacokinetically dosed busulfan. It is anticipated that the addition of e-ATG to the PC backbone already studied in 16-C-0003 will improve the poor engraftment rates seen on the IOC arm of 16-C-0003 and narrow the differences in engraftment rates between the 16-C-0003 RIC and IOC arms, resulting potentially in lower rates of graft failure for both arms of this trial as compared to even the acceptable rates seen in the RIC arm of 16-C-0003.

Patients will enroll on the IOC arm if they have defects in telomere maintenance, DNA repair, or other familial cancer syndrome where it is felt that limiting exposure to chemotherapy is important for the minimization of secondary malignancy. Additionally, patients with severe end-organ damage from their underlying disease will be eligible for the IOC arm, as their co-morbidities would prohibit use of more intensive conditioning; this will be determined by organ function, as outlined in the eligibility criteria. In both cases, it may be that the ideal conditioning, if no repair defects or organ dysfunction were present, would be more intense. However, we know that we will encounter patients in whom HCT may be the only curative approach to their disease but who are not candidates for more intensive conditioning. Thus, the IOC arm is aimed at studying if there are safe ways to perform HCT on patients who would otherwise not be

candidates for HCT, hopefully developing an approach that would extend a potentially curative therapy to patients otherwise unable to receive it.

Due to the non-random nature by which patients will be assigned to a conditioning arm, comparisons of outcomes between conditioning arms will be not performed, except for some exploratory objectives related to immune reconstitution.

1.2.6 Activity of ATG

ATG is most typically used in HCT as a method of *in vivo* T cell depleting the graft to prevent GVHD. However, there are data to suggest that, if the timing between ATG administration and the graft infusion were changed, ATG could serve as an agent to improve disease control of T-cell disorders and also improve engraftment. In a small series of 23 patients undergoing HCT for high-risk, relapsed or refractory T-cell malignancies, r-ATG (Thymoglobulin) was given proximal to the graft as GVHD prophylaxis, but with the intention of also improving disease control of the T-cell malignancy, resulting in a 2-year PFS of 70% within a group of truly high-risk T cell malignancy patients with chemotherapy refractory disease.(70) However, cGVHD rates, as typically seen using approaches other than PTCy or *ex vivo* T-cell depletion, were 30% and infectious complications were not insignificant.(70) In a clinical trial of e-ATG for the treatment of patients with NHL, including T cell lymphomas, brief responses to e-ATG were demonstrated in patients with very refractory disease, suggesting that e-ATG may provide disease control in disorders of TCP/D that have progressed to clonal T-LPDs.(71) Interestingly, ATG may also have activity against non-T cell lineages whose cell surface antigens act as epitopes to bind ATG.(72) R-ATG (ATG-Fresenius and Thymoglobulin) has been shown to induce apoptosis in not only T cells, but also B cells, monocytes, NK cells, and lymphoblastic and leukemia cell lines.(73, 74) Additionally, ATG-Fresenius spares hematopoietic stem cells, which are resistant to r-ATG mediated apoptosis.(73) Thus, e-ATG could serve to improve engraftment and also may have adjunctive activity against diseases that are T-cell malignancies and perhaps even NK-cell malignancies.

If e-ATG, when added to PC conditioning, can deepen lymphodepletion and improve engraftment, this could be a future mechanism to reduce or even eliminate the use of myelotoxic drugs such as busulfan in future studies.

1.2.7 Myeloid conditioning

Bulsulfan is a widely used as a myelosuppressive/myeloablative agent in HCT conditioning. Alternative approaches include radiation or intermediate/high doses of cyclophosphamide, although the risks of severe mucositis, GVHD, and secondary malignancy are higher with radiation as compared to busulfan and the risk of cardiac toxicity is a concern when combining higher doses cyclophosphamide with pentostatin for conditioning. Busulfan can be associated with SOS and pulmonary toxicity, but the risk of these complications can be minimized with pharmacokinetically-based, targeted busulfan dosing. In this protocol, busulfan will be added to the e-ATG-PC backbone for the RIC arm at myelosuppressive doses. In the IOC arm, busulfan will not be given.

Due to variability in individual pharmacokinetics with busulfan conditioning regimens, therapeutic drug monitoring of busulfan levels is the standard of care. Busulfan in this study will be pharmacokinetically dosed to ensure that the calculated area under the curve (AUC) of busulfan is within target range and minimize toxicities, such as SOS of the liver, which can be

associated with high AUC values.(75) Given logistical barriers in using the first busulfan dose to calculate the AUC and subsequent doses, a pre-HCT test dose of busulfan 0.8 mg/kg IV as a 2-hour infusion will be given prior to the start of the conditioning regimen, as has been successfully employed in prior studies.(76) The AUC will be calculated from the test dose and then used to calculate busulfan dosing for the conditioning with a target daily AUC value of 3600-4800 μMol-min. It is possible that unforeseen circumstances may prevent the successful completion of the test dose, whether it be that giving the dose is not feasible or the results of the blood test are not interpretable, then a default busulfan dose of 3.2 mg/kg/dose IV will be utilized.

1.2.8 Engraftment

Graft failure is primarily mediated by recipient T-cells exerting a host-versus-graft effect and thus sufficient immunodepletion of the host allows donor cells to engraft. In the setting of NMA/RIC, the clearance of residual host hematopoietic cells depends upon a graft-versus-marrow effect. Engraftment is facilitated when there are high numbers of donor T-cells and $CD34^+$ cells in the graft. In the present study, we plan to use a T-cell replete graft and will aim for 5 x 10^6 $CD34^+$ cells/kg recipient body weight from PBSC grafts to optimize engraftment. Although PBSC grafts will be the first choice to maximally reduce the risk of graft rejection, marrow grafts will be allowed as an alternative and 4.5 x 10^8 total nucleated cells (TNC)/kg recipient body weight will be the target dose.

1.2.9 GVHD prophylaxis

PTCy: PTCy will be used as the backbone of GVHD prophylaxis in this study. Johns Hopkins University has pioneered the PTCy platform for HCT, having now performed over a thousand T-cell replete transplants using PTCy, including several hundred using haplo related donors.(44) The PTCy-based platform used by Hopkins is a T-cell replete, bone marrow graft on day 0, followed by PTCy on days +3 and +4, MMF on days +5 through +35, and tacrolimus on days +5 through +180. With MAC MRD or MUD HCT using PTCy, rates of grades 3-4 aGVHD are 10%, cGVHD of 10%, and TRM of 17% at 2 years, which compare favorably to other GVHD prophylaxis approaches.(18, 77, 78) With RIC haplo HCT using PTCy for patients with hematologic malignancies, the rates of sustained donor engraftment are 87%, with grades 3-4 aGVHD of 5%, cGVHD of 15%, and TRM of 18%.(23, 79) This platform has also been associated with very low rates of EBV-PTLD, including the absence of EBV-PTLD among 785 patients transplanted at Hopkins over a 10 year period.(43) PTCy has been used in 16-C-0003 with exceptional rates of clinically significant (grade 2-4) acute GVHD and very notably no chronic GVHD to date, Figure 2. The importance of the absence of chronic GVHD cannot be understated, particularly in the setting of HCT for disorders of the immune system, where good immune function post-HCT is the ultimate goal. Given what we have seen with regard to chronic GVHD in 16-C-0003, that finding alone warrants efforts to further optimize the platform, while hopefully retaining the low rates of GVHD. If fully optimized, meaning very low rates of GVHD, graft failure, and transplant-related mortality, alongside good immune reconstitution and minimal toxicity from chemotherapy, this could be revolutionary to the field of transplant for primary immunodeficiency disease, where chronic GVHD remains a significant and greatly deleterious complication with other platforms.

Minimizing PTCy for Patients with Chemosensitivity and/or Chemotherapy Intolerance: For patients with DNA repair defects or telomere maintenance disorders, reduced doses of PTCy have successfully been used, resulting in comparable rates of engraftment and GVHD.(80, 81) Given these data, patients on the IOC arm of this trial, which will be comprised of patients with such chemosensitivity and/or significant organ dysfunction, will receive reduced doses of PTCy, given as 25 mg/kg/day on days +3 and +4 to help to minimize the direct toxic effects of the HCT platform on patients with chemosensitivity and/or chemotherapy intolerance. Patients on the RIC arm may also receive, at PI discretion, the 25 mg/kg/day dose of PTCy if they are deemed to have vulnerability/risk of increased toxicity from excessive chemotherapy, but are also deemed to need RIC level conditioning to optimally control their disease entering HCT.

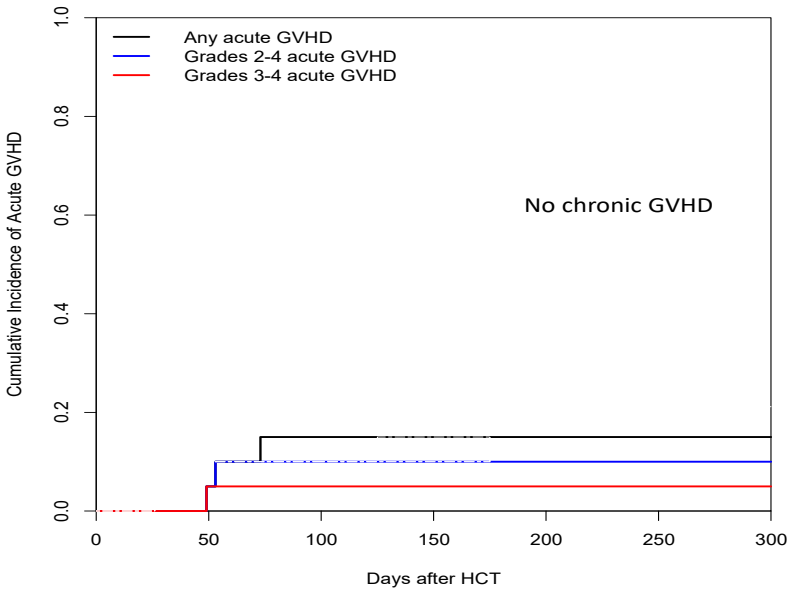


Figure 2. Cumulative incidence of acute GVHD for the RIC arm of 16-C-0003.

Adjuncts to PTCy: In the RIC/IOC setting, agents in addition to PTCy are necessary for GVHD prophylaxis. Most commonly, this has been the addition of MMF (typically day +5 through +35) and tacrolimus (typically day +5 through day +60-180, depending on transplant center). Calcineurin inhibitors such as cyclosporine or tacrolimus inhibit T-cell receptor signaling and can impede tolerance induction. However, as alternatives such as the mammalian target of rapamycin inhibitor sirolimus may favor mixed T cell chimerism and normalize the function of host T cells for patients who have activating mutations in the PI3k/Akt/mTOR pathway, patients in on this protocol will receive tacrolimus from days +5 through +90 post-HCT. In 16-C-0003, we have had two patients with activating PI3k mutations show worsening T cell chimerism on sirolimus post-HCT, followed by improvement in T cell chimerism upon sirolimus cessation. Thus, it is felt that sirolimus and its associated mixed chimerism that follows early post-HCT will not be well tolerated by patients whose host T cells may have an advantage.

Tacrolimus Duration: Johns Hopkins has published the results of a prospective study that evaluated the incidence of GVHD, graft failure, relapse, and NRM, as well as survival endpoints, when the duration of post-HCT tacrolimus was shortened from stopping on day +180, to day +120, to day +90, to day +60.(82) With improved 1-year GRFS in the tacrolimus through day +60 arm, Johns Hopkins has transitioned to stopping tacrolimus in patients at day +60 after HCT, provided there is no grade 2-4 acute GVHD, donor T cell chimerism is at least 5%, and there is no relapse.(82) Based on these data, it appears that outcomes can be optimized, even within non-malignant diseases, if immunosuppression duration is shortened. In the Hopkins study, there was a signal for more grade 2 acute GVHD between days +60 and +90, although no increase in grade 3-4 acute GVHD, chronic GVHD, or TRM.(82) In another study by Hopkins, grade 2 acute GVHD was associated with improved PFS in patients with hematologic malignancies receiving

haploidentical HCT, contributing to data in the matched donor setting that grade 2 aGVHD may further improve the efficacy of HCT through an associated graft-versus-tumor effect without introducing additional morbidity from more clinically significant acute GVHD.(83, 84) Because there is no benefit to any GVHD in non-malignant diseases, apart from the initial graft-versus-host marrow effect that promotes engraftment early post-HCT, we will continue tacrolimus through day +90 to be particularly conservative.

MMF Duration: Per the Hopkins GVHD prophylaxis approach for RIC transplants, MMF was empirically added to theoretically promote engraftment and prevent acute GVHD, but the necessity of MMF for these purposes is not well studied. However, other transplanters throughout the world have performed RIC, alternative donor transplants using PTCy as GVHD prophylaxis and have omitted MMF from the regimen with rates of GVHD and graft failure that are no different than with PTCy platforms that contain MMF.(85, 86) Shortening the duration of MMF was motivated by a desire to further improve count recovery, immune reconstitution, and lessen viral complications, namely BK-associated cystitis, which is very common post-HCT in our primary immunodeficiency disease patients on 16-C-0003. There are multiple side effects of MMF, including severe lymphopenia, which likely slows immune reconstitution and contributes to issues with cell-mediated immune control of viruses, as well as gastrointestinal upset and diarrhea.

In 16-C-0003, MMF is currently continued through day +18 on the RIC-MMF arm for recipients of mismatched related or unrelated grafts, and no longer used at all for recipients of matched related or unrelated grafts given no increased events of graft failure, GVHD, or death with duration de-escalation in the latter cohort. If there are no increased events of graft failure, GVHD, or death in the mismatched cohort for the first 6 patients, it will eventually be omitted completely from the post-HCT immunosuppressant regimen for recipients of mismatched grafts as well on 16-C-0003. To be conservative in the MMF duration in the current protocol, balancing both the desire to limit MMF exposure because of the toxicities and intolerances discussed above, acknowledging that the duration de-escalation study is still ongoing in 16-C-0003, and that peripheral blood stem cell grafts in the current study may be associated with greater GVHD incidence, patients on this protocol will receive MMF from day +5 through +25 and it will be stopped on day +25 once initial chimerism results are available and the presence of donor chimerism is confirmed. For most patients, count recovery enabling initial assessment of chimerism occurs first between days +14-22, allowing a chimerism assessment to be sent and resulted by day +25.

1.2.10 Rationale for subsequent donor cell infusions

While mixed or split donor chimerism *may* be sufficient to reverse the disease phenotype in patients with disorders of TCP/D, mixed chimerism may be a harbinger of graft failure in these diseases and may also be a risk for disease relapse. The use of RIC/NMA conditioning regimens are associated with increased risk of mixed chimerism and therefore graft failure, as compared to MAC approaches. However, the merits of these less intensive approaches should not be underestimated, as long as the T cell chimerism, and resulting myeloid chimerism, can be secured and maintained post-HCT. In the present study, mixed/split donor chimerism may be managed with the administration of donor cell infusion (DCI) in the post-HCT period, most typically a CD3-aliquoted lymphocyte-DCI. Patients who, on serial sorted chimerism assessment, have mixed donor chimerism that is thought to be on a trajectory that will threaten

the persistence of the graft and/or contribute to disease relapse (non-malignant or malignant) will be eligible to receive a DCI. DCI has been shown previously to be associated with improved chimerism, ameliorated graft function, and a graft-versus-lymphoma effect.(87-90)

1.2.11 Necessity of the NIH Environment to Conduct this Clinical Trial

As TCP/D disorders are rare and the patients require the input of specialists across the fields of transplantation, hematology/oncology, immunology, genetics, and infectious disease, among others, clinical investigations of HCT for TCP/D are well-suited for the NIH, where the collective expertise and resources exist to study and treat these diseases. Although HCT is the sole potentially curative therapy for many disorders of TCP/D, there remains a paucity of clinical research and experience in the transplantation of many of these diseases, in large part due to the rarity of the diseases, difficulty with pre-HCT disease control, and the frequency of co-morbidities that make most HCT approaches prohibitive for many such patients. The ongoing 16-C-0003 trial has led to the referral of many patients to the NIH with rare diseases that are studied by investigators throughout the NIH. Thus, protocols offering a potential therapy for patients with rare diseases that are studied on natural history studies here at NIH contribute to our ability to bring patients with diseases of interest to NIH to further research endeavors across institutes.

Mechanisms of PTCy and Ongoing Work at NIH: PTCy was first shown in murine allograft models to induce immune tolerance and prevent GVHD.(91) The mechanisms by which PTCy prevents GVHD are still being elucidated in work done by Dr. Christopher Kanakry, a Lasker Scholar and tenure-track investigator within the CIO (formerly ETIB) of the NCI.(25) Building on previous data establishing that the high expression of aldehyde dehydrogenase (ALDH) in hematopoietic stem cells spares these cells from the cytotoxic effects of cyclophosphamide,(92) Kanakry and colleagues have shown that regulatory T cells, which are key mediators of tolerance induction and GVHD prevention, also express high ALDH and are therefore resistant to PTCy.(25) Dr. Chris Kanakry's translational research is focused on further understanding the mechanisms of PTCy, as well as how to optimize PTCy dosing and timing so as to effectively prevent GVHD but also enable the incorporation of novel therapies post-HCT. Patient specimens from this study will be used in correlative studies outlined in this protocol (Section **5.1**) and will contribute to his ongoing work related to PTCy.

1.2.12 Summary

It should be emphasized that the focus on immunodepletion rather than myeloablation has only very recently come to the forefront of the HCT field, and thus clinical research that investigates ways to optimize and improve these reduced-intensity, toxicity-sparing approaches is critical to advancing the field. Approaches that can result in safer, less toxic transplants and produce favorable outcomes and disease control, even in diseases that are hard to engraft and/or are associated with high rates of post-HCT relapse, are needed. The approach to immunodepletion using serotherapy solely as host conditioning and not also as GVHD prophylaxis has not been studied and has not been coupled with highly effective approaches to GVHD prophylaxis such as PTCy-based platforms. Thus, the PTCy platform, with further tailoring of the immunosuppressive regimen as detailed above, seems ideally suited to be combined with host-only targeted serotherapy to maximize engraftment, disease control, and GVHD prevention, as well as retain the good immune reconstitution seen with PTCy.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria - Recipient

2.1.1.1 Age ≥ 4 years

2.1.1.2 TCP/D deemed to be of sufficient past severity to warrant HCT that meets at least one of the criteria below:

- Identified germline T-cell activating mutation in the PI3k pathway
- Identified ADA2 deficiency (biallelic mutations in *CECR1 (ADA2)* and/or phenotypically with low ADA2 level) leading to neutropenia requiring chronic GCSF therapy or to transfusion-dependent anemia or thrombocytopenia
- T-cell infiltration of liver, spleen, lymph nodes, marrow, lungs, gut, or other organs by T cells, as evidenced by laboratory, radiographic, and/or anatomic pathology evaluation, resulting in organ dysfunction and/or organomegaly
- Latent herpesvirus infection in T lymphocytes
- History of or active evidence of hemophagocytic lymphohistiocytosis (HLH)
- Recurrent or prolonged fevers attributed to immune dysregulation
- T-cell population in blood and/or marrow with immunophenotype of large granular lymphocytes (LGL), with or without clonality or lymphocytosis
- T-cell lymphoproliferative disorder in the setting of an underlying immune defect
- Immune-mediated cytopenias of one lineage requiring transfusion or GCSF support or of 2 or 3 lineages with or without transfusion or support
- Chronic active EBV

2.1.1.3 At least one potential 7-8/8 HLA-matched related (excluding an identical twin) or unrelated donor (at HLA-A, -B, -C, and -DR), or an HLA-haploidentical related donor, based on initial low resolution unrelated donor search and/or at least one biologically-related family member who has at least a 25% chance of being at minimum an HLA-haploidentical match and is potentially suitable to donate based on reported family history. HLA typing of potential donors and/or mutation testing does not need to be completed for eligibility.

2.1.1.4 Adequate end-organ function, as measured by:

- Left ventricular ejection fraction (LVEF) ≥ 40% by 2D echocardiogram ECHO, or left ventricular shortening fraction ≥ 20% by ECHO for subjects receiving RIC, or LVEF ≥ 30% if the subject has radiologic evidence of aortic, renal, or coronary artery vasculitis. LVEF ≥ 30% for subjects receiving IOC.
- Pulmonary function tests: DL_{co} (corrected for hemoglobin) and $FEV_1 \geq 40\%$ of predicted for the RIC arm, and ≥ 30% predicted for the IOC arm; or in pediatric subjects, if unable to perform pulmonary function tests, there should be no evidence of dyspnea at rest, no requirement for supplemental oxygen, and oxygen saturation >92% on room air. Calculations will be based on the values reported in CRIS.

- Bilirubin ≤ 3.0 mg/dL (unless due to Gilbert's syndrome or hemolysis) for subjects receiving RIC and bilirubin ≤ 5.0 mg/dL for subjects receiving IOC (unless due to Gilbert's syndrome or hemolysis); ALT and AST ≤ 5 x ULN for subjects receiving RIC or < 10 x ULN for subjects receiving IOC. Subjects who are above these bilirubin, ALT, or AST thresholds may be eligible for the RIC or IOC arm if evaluated by a hepatologist who deems the liver function test abnormalities to be potentially reversible with HCT.
- Estimated creatinine clearance of ≥ 50 mL/min/1.73 m^2, calculated using eGRF in the clinical lab for adults and the Schwartz formula (see **Appendix E**) for pediatric subjects, if eGFR not reported by the clinical lab.

2.1.1.5 Karnofsky (adults) or Lansky (children) performance status of ≥ 50% or ECOG performance status of 2 or less for the RIC arm and ≥ 30% or ECOG performance status of 3 or less for the IOC arm (see **Appendix A**)

2.1.1.6 Ability of subject or parent/legal guardian or Legally Authorized Representative (LAR) (e.g., in cases of adults unable to consent) to understand and the willingness to sign a written informed consent document

2.1.1.7 Not pregnant or breastfeeding. As therapeutic agents used in this trial may be harmful to a fetus, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for at least one-year post-allo HCT. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in the study, she should inform her treating physician immediately.

2.1.1.8 Disease status: Subjects with lymphoproliferative disorder (LPD), LGL, HLH, or other TCP/D disorders requiring standard therapies to prepare for HCT should be referred in remission if possible. However, these diseases are often aggressive and require swift evaluation for HCT while concurrently attempting to establish disease control through the administration of standard therapies. If ongoing therapy for the underlying disease outside of the NIH is not in the best interest of the subject according to the clinical judgment of the PI, then the subject may receive standard treatment for his/her underlying TCP/D disorder as a bridge to HCT on this protocol, prior to starting the research phase of the study. If it becomes apparent that the subject will not be able to proceed to HCT, then he/she must come off study. Subjects receiving standard therapy will be told about the therapy, associated risks, potential benefits, alternatives to the

proposed therapy, and the availability of receiving the same treatment elsewhere, outside of a research protocol.

2.1.2 Exclusion Criteria – Recipient

2.1.2.1 Subjects who are receiving any other investigational agents, with the exception of virus-specific cytotoxic T-cells for the treatment of viral infection/reactivation prior to allo HCT.

2.1.2.2 Prohibitive allergy to a study drug or to compounds of similar chemical or biologic composition of the agents (e-ATG, steroids, cyclophosphamide, busulfan, pentostatin, tacrolimus, MMF, G-CSF) used in the study.

2.1.2.3 Active psychiatric disorder which is deemed by the PI to have significant risk of compromising compliance with the transplant protocol or which does not allow for appropriate informed consent

2.1.2.4 HIV positive or other acquired immunodeficiency that, as determined by the PI, interferes with the assessment of TCP/D severity and/or the attribution of clinical manifestations of immunodeficiency to a disorder of TCP/D.

2.1.2.5 MAGT1 mutation and active need to take anti-platelet agents and/or therapeutic anti-coagulation that cannot be interrupted during aplasia

2.1.2.6 Lack of adequate central venous access potential

2.1.3 Inclusion Criteria – Related Donor

2.1.3.1 Age ≥ 4 years

2.1.3.2 Related donor deemed suitable and eligible, and willing to donate, per clinical evaluations who are additionally willing to donate blood, urine, and marrow specimens for research. Related donors will be evaluated in accordance with existing Standard Policies and Procedures for determination of eligibility and suitability for clinical donation. Note that participation in this study is offered to all related donors, but is not required for clinical donation, so it is possible that not all related donors will enroll onto this study.

2.1.4 Exclusion Criteria – Related Donor

2.1.4.1 None.

2.1.5 Inclusion Criteria – Unrelated Donor

2.1.5.1 Unrelated donors will be evaluated in accordance with existing NMDP Standard Policies and Procedures, available at: http://bethematch.org/About-Us/Global-transplant-network/Standards/, except for the additional requirement of EBV serostatus testing for clinical purposes of donor selection. Note that participation in this study is offered to all unrelated donors but not required for clinical donation, so it is possible

that not all unrelated donors will enroll on this study. Unrelated donors only enroll if they contribute research specimens, which is optional.

2.1.6 Exclusion Criteria – Unrelated Donor

2.1.6.1 Unrelated donors: failure to qualify as a National Marrow Donor Program (NMDP) donor per current NMDP Standards, available at: http://bethematch.org/About-Us/Global-transplant-network/Standards/. Exceptions to donor eligibility (e.g. foreign travel, tattoos) do not automatically exclude the donor and will be reviewed by the PI.

2.1.7 Recruitment Strategies

Information about this protocol will be posted on www.clinicaltrials.gov, the NIH Clinical Center website, NIH social media forums and the NCI Patient Recruitment website.

2.2 SCREENING EVALUATION

2.2.1 Screening Activities Performed Prior to Obtaining Informed Consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver for these activities is requested in section **10.5.4.**

2.2.2 Screening Activities Performed After A Consent for Screening Has Been Signed

The following activities will be performed only after the recipient has signed the consent for this study for screening. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

2.2.2.1 Recipient

2.2.2.1.1 Performed on the recipient at any time prior to initation of study therapy

- Evaluation of central venous access potential
- Testing to determine the availability of a suitable donor and to aid in donor selection: High resolution HLA typing at HLA-A, -B, -C, -DR, and –DQ loci by the Department of Transfusion Medicine (additional HLA typing must be performed twice at any time in the two years prior to donor cell collection); anti-donor HLA antibody screen (only necessary for subjects with less than 10/10 HLA matched donors or recipients of prior allogeneic cell infusions); blood typing (ABO/Rh); EBV and CMV serostatus (anti-EBV antibody panel, CMV IgG and IgM, EBV early antigen). Results may be pending at the time of second enrollment onto study therapy.

- Confirmation of a diagnosis of a disorder of TCP/D. Bone marrow biopsy slides that are available should be reviewed in addition to surgical pathology slides. Subjects will have their diagnosis confirmed by relevant history, laboratory, imaging, and pathology findings. If there is an identified germline mutation for a subject with a disorder of TCP/D, the mutation report will be required. Those with mutational testing but no identified mutation should have their mutation reports obtained. These confirmations of disease may be performed at any time prior to or during screening evaluation.

2.2.2.1.2 Performed on the recipient within the four weeks prior to initiation of study therapy.

- History and physical exam, including weight, height, and vital signs
- Assessment of performance status using Karnofsky (adult) or Lansky (pediatric) scales as applicable; or ECOG performance status
- To assess for disorders of TCP/D: Unilateral bone marrow aspiration and biopsy, with studies appropriate to evaluate for a primary marrow disorder and/or hematologic malignancy, including immunohistochemistry, *in situ* hybridization, flow cytometry, molecular testing for at least T and B clonality, and cytogenetics, as appropriate. Bone marrow biopsy may be deferred until baseline evaluations, at the discretion of the PI, if the patient has a confirmed diagnosis of a disorder of TCP/D from prior evaluations (marrow biopsy or otherwise), making a recent marrow unnecessary for eligibility purposes.
- Antibody screen for HIV-1/2 and quantitative HIV-1 RNA PCR
- 2D echocardiogram (may be performed within 12 weeks of second enrollment onto study therapy, if no cardiotoxic drugs have been given in the interim; repeat after cardiotoxic drug, if administered in the interim)
- Pulmonary function testing, including DLco and FEV_1, or assessment of oxygenation on room air for pediatric subjects unable to undergo pulmonary function testing (may be performed within 12 weeks of second enrollment onto study therapy)
- Assessment of renal function - acute care panel, including sodium, potassium, chloride, bicarbonate, creatinine, glucose, and urea nitrogen; mineral panel, including phosphorus, magnesium, albumin, and calcium
- Assessment of hepatic function - hepatic panel, including alkaline phosphatase, aspartate transaminase, alanine transaminase, total bilirubin, and direct bilirubin
- β-hcg pregnancy test (serum or urine) on all women of childbearing potential
- If clinically indicated, a biopsy of any concerning lesions found during the course of evaluations will be done to evaluate for malignancy
- If clinically indicated, CNS imaging and/or lumbar puncture to evaluate for CNS involvement by malignancy
- Review of current medications, including other investigational agents, and allergies

2.2.2.2 Related Donors

- Multiple potential donors may be screened concurrently. Donor screening will occur on the screening portion of 18-C-0135 or another suitable protocol.
- Donor eligibility and suitability will be determined by a licensed independent provider according to the most recent AABB regulations and standards and NIH CC guidelines.

The selected donor will be enrolled on the research portion of the study to contribute research specimens, if he/she consents to this.

- The clinical, laboratory, and radiologic assessments that will be performed on related donors are as necessary to determine eligibility and suitability and are independent from this research protocol.

2.2.2.3 Unrelated Donors

- Donor eligibility will be determined by the NMDP-affiliated donor center physician according to the most recent FDA, NMDP, and AABB regulations and standards
- Exceptions to donor eligibility (e.g. foreign travel, tattoos) which do not automatically exclude the donor will be reviewed by the PI. The recipient will be informed and consented to any exceptions which could potentially increase the risk of their transplant.
- The clinical, laboratory, and radiologic assessments that will be performed on unrelated donors by the NMDP donor center are outlined on the NMDP Standards website at: http://bethematch.org/About-Us/Global-transplant-network/Standards/.
- Donor testing for EBV serostatus (anti-EBV antibody panel and EBV early antigen) will be required for unrelated donors, as this information is necessary to inform decisions regarding donor prioritization, as outlined below.

2.2.3 Donor Selection and Prioritization

In the event that two or more eligible and suitable donors are identified, the following order of priority for donor selection will be used:

- HLA
  In order of priority:
  - HLA-matched related donor (7-8/8 at HLA-A, -B, -C, and -DR loci)
  - HLA-matched unrelated donor (8/8 at HLA-A, -B, -C, and -DR loci) or HLA-haploidentical related donor, with the choice between these two donor sources left to the discretion of the PI based on other factors
  - HLA-matched unrelated donor (7/8 at HLA-A, -B, -C, and -DR loci)
- ABO
  In order of priority:
  - ABO cross-match compatible
  - Minor ABO incompatible
  - Major ABO incompatible
- CMV serostatus: CMV negative donor preferred if recipient is CMV negative; CMV positive donor preferred if recipient is CMV positive
- EBV serostatus: EBV negative donor preferred if recipient is EBV negative; EBV positive donor is preferred if recipient is EBV positive
- Sex
  In order of priority:
  - Male
  - Nulliparous female
  - Multiparous female

Other factors such as donor age and health history will be integrated into the donor selection process and may be prioritized over HLA, ABO, CMV serostatus, EBV serostatus, and sex.

Prioritization of donors beyond those listed above are suggested but not mandated by the protocol.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

2.3.1 Protocol Entry

The participant's entry date on protocol is considered to be the day that consent form has been signed by the recipient. The treatment start date is considered to be the day the recipient begins his/her conditioning chemotherapy.

2.3.2 Registration

Unrelated donors who contribute research specimens will be registered with limited demographic information only.

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.3 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a reversible organ dysfunction, inadequate donor options, and/or lack of disease severity at the time of initial screen may be rescreened should there be changes that could render the individual eligible on re-screen.

2.3.4 Treatment Assignment and Randomization/Stratification Procedures for Registration Purposes Only

Cohorts

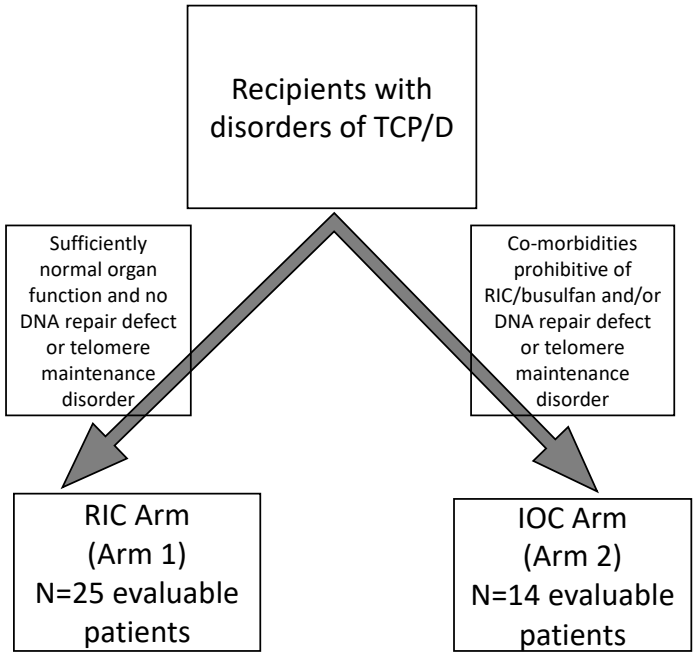
Number	Name	Description
1	*TCP/D Disorder HCT Recipients*	*Recipients age 4 years and older with disorders of TCP/D*
2	*HCT Donors*	*7-8/8 HLA-matched related or unrelated donor, or an HLA-haploidentical related donor*

Arms

Number	Name	Description
1	*RIC Arm*	*RIC+allo HCT+GVHD prophylaxis*
2	*IOC Arm*	*IOC+allo HCT+GVHD prophylaxis*
3	*Donor Arm*	*Donors for Recipients in Arm 1 or Arm 2*

Randomization and Arm Assignment

There is no randomization. There will be two arms in this protocol that vary in the intensity of the conditioning regimen (RIC or IOC). The two arms are shown below.



Although conditioning arm assignment guidelines are outlined in the protocol, the conditioning arm assignment for each recipient may ultimately deviate from the outlined strategy at the discretion of the PI such that the PI can deem a recipient only eligible for the IOC arm even if meeting RIC arm eligibility criteria, based on factors related to an individual recipient's clinical history, extent of prior therapies, organ function, comorbidities, disease status, etc. It is also possible that a participant, after meeting IOC arm eligibility, may be deemed eligible by the PI for the RIC arm, based on organ function, disease status, etc.

Recipients in cohort 1 will be assigned to either Arm 1 or Arm 2 based on organ function and chemosensitivity. If a subject has been registered on the RIC arm, but has changes in organ function that make the subject only eligible for the IOC arm and the plan is to proceed with treatment on the IOC arm. If a subject has been registered for the IOC arm but has changes that make the subject eligible for the RIC arm and the plan is to proceed with treatment on the RIC arm.

Subjects in cohort 2 will be assigned to arm 3 as donors.

Arms can enroll concurrently and pauses in accrual that apply to one arm do not apply to other arms. Stopping rules will apply to each arm separately.

2.4 BASELINE EVALUATION

Subjects (recipients) who receive therapies after screening and before protocol therapy (HCT) that have potential cardiac toxicity (such as anthracyclines) or pulmonary toxicity (such as bleomycin) should have ECHO or PFTs repeated, respectively, to confirm that eligibility criteria are still met prior to proceeding to protocol therapy (HCT). Liver and kidney function should

also be confirmed to still meet eligibility criteria if a subject receives standard of care therapy after screening and before HCT.

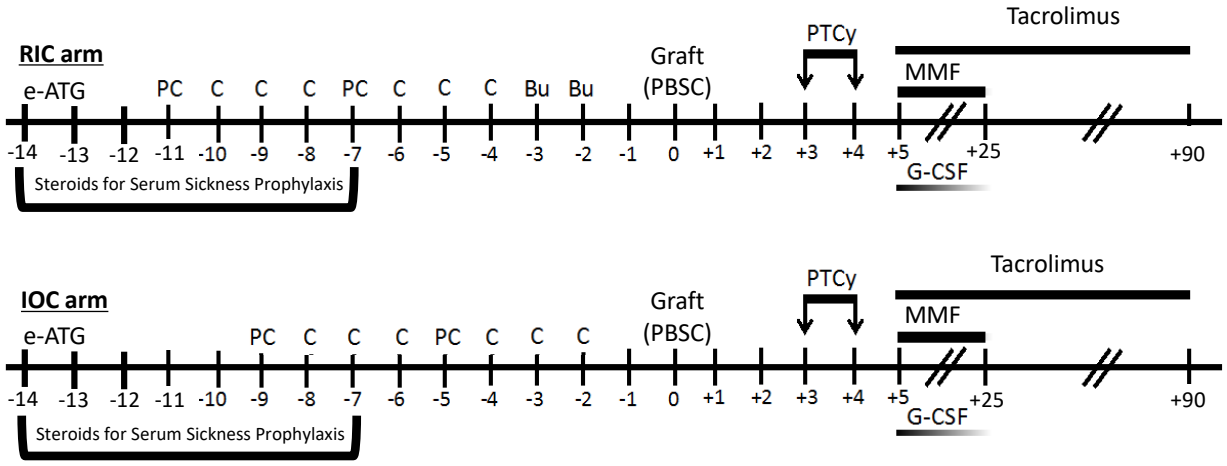
The following baseline evaluations and referrals should be scheduled after eligibility is confirmed and ideally performed in the four weeks prior to the start of HCT conditioning. Tests performed prior to eligibility confirmation may not need to be repeated during the baseline evaluations at the discretion of the PI. If a recipient has received interim therapy prior to protocol therapy (HCT) as a standard of care bridge to HCT, some baseline evaluations may need to be repeated after standard of care treatment completion, at the discretion of the PI.

- Vital signs, weight, performance status assessment
- Antibody screens (IgG ± IgM) for hepatitis A, B, and C virus, HTLV-I/II, T cruzi, CMV IgG and IgM, anti-EBV antibody panel and EBV early antigen, HSV I/II, VZV, West Nile virus, and Toxoplasmosis; syphilis screen per current methodology or institutional practice
    - Recipients on chronic IgG replacement may need viral PCRs in lieu of or in addition to antibody testing, as clinically indicated and available, as well as specifically timed serologic testing to minimize immunoglobulin replacement interference with serology results
- PCR in blood for EBV, CMV, JCV, BK virus, adenovirus, HHV6, and Toxoplasmosis
- PCR in urine for BK virus, adenovirus, and JC virus
- Complete blood count with differential and platelet count, lymphocyte phenotype panel (TBNK), peripheral blood smear, peripheral blood molecular analysis of T and B cell clonality
- Total protein, lactate dehydrogenase (LDH), uric acid, reticulocyte count, quantitative immunoglobulins (IgG, IgA, IgM), IgE, fasting lipid profile with triglycerides, iron and transferrin panel
- PT, PTT, fibrinogen
- Cytokine panel, CRP, ESR, ferritin
- Isohemaglutinin titer if donor and recipient are ABO mismatched, except for recipients with type AB blood type
- Glucose-6-phosphate dehydrogenase deficiency screening, hemoglobin electrophoresis (need not be repeated if performed prior to registration)
- Gamma glutamyl transferase
- Fasting iron studies
- Urinalysis with microscopic examination, spot urine protein to creatinine ratio
- For females of child-bearing potential: serum or urine βHcg
- EKG
- PCR testing of DNA mini-satellite regions for future determination of chimerism by the Department of Laboratory Medicine (can be performed at screening or any time before HCT)
- QuantiFERON TB Gold testing for tuberculosis (need not be repeated if performed in the prior 3 months)
- Imaging as clinically indicated, based on the recipient's clinical history and current medical condition.

- Dental consultation, or dental records indicating adequate dental evaluation and management in the 6 months prior to HCT
- Social work consultation
- Nutrition assessment (initial consult)
- Transplant Infectious Disease consultation
- For recipients with MAGT1 mutation, to evaluate for risk of epistaxis and plan for post-HCT transfusion support: ENT consult, DTM consult, hematology evaluation as appropriate and possible based on platelet count, imaging of the nose/sinus/base of skull
- Endocrinology laboratory evaluation, with or without consultation as appropriate. Labs for post-pubertal recipients: thyroid stimulating hormone (TSH), free T4, total T3, adrenocorticotropic hormone (ACTH), 8 am cortisol, 25-hydroxy vitamin D, 1,25-hydroxyvitamin D, parathyroid hormone (PTH), males only - 8 am testosterone (free and total); hemoglobin A1c, fasting glucose, follicle stimulating hormone (FSH), luteinizing hormone (LH), females only – estradiol. Pre-pubertal recipients will have age-appropriate labs drawn.
- HCT-CI Score, using online calculator at: http://www.hctci.org/Home/Calculator
  Note that only laboratory values prior to the start of conditioning should be used. Laboratory values from the following timeframes should be used: days -24 to -14. If only one set of laboratory values are available after day -24, older laboratory values may be used as the second set of values, per HCT-CI guidelines.
- Research sample collection (See table in Section **5.1.1**)
  In recipients receiving standard of care bridge therapy, depending on logistics and disease status, pre-HCT research sample collection may be performed before or after standard of care therapy. Timing will be determined based on how the planned standard of care therapy might impact upon the research labs and underlying research objectives.
- Review of current medications

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN



See Section **3.3.2** for conditioning regimen dosing information. See Section **3.3.3** for GVHD prophylaxis dosing information.

3.2 DONOR STEM CELLS – PREFERRED GRAFT SOURCE AND GRAFT CHARACTERISTICS

PBSC grafts are the preferred graft source for this protocol. If the donor is unwilling or unsuitable to donate PBSCs, marrow can alternatively be used, at the discretion of the PI. The grafts will not be manipulated to deplete T cells. Processing for ABO incompatibility will follow DTM practices. Donor lymphocytes may be collected for the standard clinical practice, with cryopreservation by DTM and potential future clinical use (dedicated donation to the recipient). The number of donor lymphocytes aliquoted and cryopreserved will depend on the counts obtained from the clinical stem cell collection/donation. Collecting and cryopreserving donor lymphocytes for future clinical use facilitates the timeliness in which a recipient in need of a donor lymphocyte infusion can receive the therapy. Donor lymphocytes will be collected and stored as outlined in the DTM protocol-specific instructions. Additional collections of donor lymphocytes from unrelated donors will not be done in an anticipatory manner, although unrelated donors will be advised that there is a higher than typical chance of needing a second collection, given that recipients on this protocol are deemed high risk for graft failure.

The target bone marrow dose will be at least 4.5 x 10^8 TNC/kg of the recipient's ideal body weight. The minimum graft dose, calculated to help inform if the donor is of adequate size to donate for the recipient, should be 2.5 x 10^8 TNC/kg of the recipient's ideal body weight. The target PBSC collection is 5 x 10^6 $CD34^+$ cells/kg of the recipient's ideal body weight and the minimum graft dose is 2.5 x 10^6 $CD34^+$ cells/kg for PBSC collection. Most apheresis procedures will yield the target PBSC collection. If the target collection of 5 x 10^6 $CD34^+$ cells/kg of the recipient's ideal body weight is not met on day 5, a second day of apheresis may be performed at the discretion of the PI and depending on donor tolerability. Target doses are meant to guide collection planning, not to suggest a minimum or maximum cell dose for the recipient.

The stem cell product will be infused fresh in the majority of cases. Cryopreservation of stem cell products is permitted if necessary for logistical reasons but discouraged. If the PBSCs must be collected more than 24 hours prior to the infusion of cells on the day of HCT, the PBSC product will be cryopreserved by the DTM per standard procedures. PBCSs can be stored at 4-8^{o}C for up to 24 hours. The stem cell product will be cryopreserved by methods currently in use and detailed in the CPS DTM standard operating procedures and stored in a liquid nitrogen storage tank. The number of $CD34^{+}$ cells and $CD3^{+}$ in each cryopreserved bag will be calculated. Viability of the product will be assessed at the time of use. Any surplus cells that have been collected may be released for research at the PI's discretion.

For pediatric donors, research sample collection is limited to up to a collective total of 10 mL of blood/marrow/peripheral blood stem cells, collected at the time of clinical donation, for research purposes.

3.2.1 Bone marrow harvest procedure – Unrelated Donors

URD Marrow Donors will undergo bone marrow harvest at NMDP affiliated collection centers according to NMDP Standards. A target dose of 3-5 x 10^8 Total Nucleated Cells/kg will be requested. Based on NMDP Collection Center standards, total volume of marrow to be collected from donor ranges from 15-20 mL/kg based on donor weight. Discrepancies in donor and recipient weight may predict a lower cell dose yield and will be examined on a case by case basis. Bone marrow products are couriered to the NIH by trained couriers and infused within 48 hours of collection.

3.2.2 Peripheral Blood Stem Cell Mobilization and Collection – Unrelated Donors

URD PBSC Donors will undergo mobilization and leukapheresis at NMDP affiliated apheresis centers according to NMDP standards. A target dose of 5-8 x 10^6/kg CD34 cells will be requested. URD PBSC products are couriered to the NIH by trained couriers and infused within 72 hours of collection. If the target dose is exceeded, the excess cells will be cryopreserved per the Protocol Specific Instructions in DTM.

3.3 DRUG ADMINISTRATION

Pre-HCT Standard of Care Bridge Therapies

- Given the potential aggressive nature of the diseases that recipients transplanted on this trial will have, it may be necessary to treat potential HCT recipients with standard of care therapies as the screening evaluations and donor search are being completed.
- If given standard of care therapy, potential recipients will be removed from study when/if it is determined that a suitable donor cannot be found or that the recipient will not be eligible to move forward with HCT. The potential recipient will then be transitioned back to his/her home doctor for ongoing treatment and/or palliation or enrolled in another NIH trial, if appropriate.
- The standard of care bridge therapies will be solely aimed at improving disease control pre-HCT to minimize the chance of relapse after HCT and/or to improve disease-associated organ dysfunction to above the threshold necessary for eligibility.
- Standard of care therapies include immunosuppressant drugs, serotherapy, targeted agents, chemotherapeutics, and biologics published in peer-reviewed journals that have

been used to treat like disorders, such as aplastic anemia, hemophagocytic lymphohistiocytosis, chronic active EBV, and autoimmune cytopenias.

- If a recipient was initially enrolled on the RIC arm, but has a decrease in organ function after standard of care bridge therapy such that the recipient becomes only eligible for the IOC arm, this change will be discussed with the recipient and, if the decision is to move forward, the recipient will be treated on the IOC arm and the treatment arm will be updated with the registration office.
- If the recipient, in the course of standard of care bridge therapy, becomes ineligible to move forward to HCT, the recipient will be taken off study at that time and will transition back to his/her home doctor.
- The follow-up evaluations and study calendar does not apply during this time period. During this time period, pre-HCT research specimens may be obtained, as outlined in the **Table 1** and **Table 2**
- Stopping rules do not apply during this time period.

3.3.1 Pre-HCT Busulfan Test Dose

- Recipients on the RIC arm will receive busulfan as part of the conditioning regimen. A test dose of busulfan 0.8 mg/kg IV as a 2-hour infusion will be given prior to the start of the conditioning regimen in order to calculate the AUC.
- Recipients will require a central venous catheter for administration of the busulfan test dose.
- The busulfan dose used in the conditioning regimen will be determined based on the calculated AUC of the test dose or real time pharmacokinetics (PKs). The targeted daily systemic exposure of busulfan will be 4600 µMol-min, with an acceptable range 3600-5600 µMol-min.
- Seizure prophylaxis is not needed for the busulfan test dose.

The purpose of the busulfan test dose is to obtain pharmacokinetic blood samples of busulfan for calculation of an AUC. The calculated AUC of the test dose (performed by specialized pharmacists in the Clinical Center) will then be utilized to dose busulfan for the conditioning regimen based on a targeted busulfan AUC. For recipients age > 16 years, busulfan dosing will be based on ideal body weight (IBW), as defined below, or actual body weight, whichever is lower.

Ideal Body Weight (IBW)	Definitions
IBW (adult male, age > 20)	50 kg + 2.3 kg per inch over 5 feet
IBW (adult female, age > 20)	45.5 kg + 2.3 kg per inch over 5 feet
IBW (pediatrics, age 4-20)	Weight based on same percentile as stature for age using CDC weight for stature growth curves

For adult recipients who are greater than 120% of IBW, busulfan will be dosed on an adjusted ideal body weight (ideal body weight plus 25% of the difference between ideal and actual weight). For children (age 4 through 16), busulfan dosing will be based on actual body weight, although the ideal body weight may be used for the test dose if it is lower than the recipient's actual body weight. Four busulfan blood samples will be drawn following the administration of the test dose

in a green top (sodium heparin) collection tube (1 mL specimen volume). Optimally, samples should be obtained from a peripheral vein in the arm opposite to the central line where busulfan is infused. If a peripheral vein sample is not feasible, the samples should be obtained from a different central catheter lumen than that used for the busulfan infusion. The first specimen should be drawn immediately after termination of the 2-hour intravenous infusion of busulfan. Additional specimens should also be drawn at 1 hour, 2 hours and 4 hours after termination of infusion. Each sample should be placed on ice immediately after collection. Antiemetic prophylaxis will be administered for the busulfan test dose, but anti-seizure prophylaxis will not be required. The test dose may be skipped if real-time busulfan PKs are done during conditioning.

The busulfan dose utilized in the conditioning regimen will be determined based on the calculated AUC of the test dose or real time PKs. This targeted daily systemic exposure of busulfan is 4600 µMol-min (range 3600-5600 µMol-min). The range of the target AUC allows for adjustment at the discretion of the PI to minimize toxicities based on individual recipient factors and organ function. The busulfan dose for conditioning will be calculated using the following equation:

$$\text{Busulfan dose} = \frac{\text{Target AUC of 3600-5600 µMol-min x Test dose (mg)}}{\text{Test dose AUC (µMol-min)}}$$

If the test dose or real time PKs cannot be completed for unforeseen reasons or if the pharmacokinetic data obtained cannot be accurately interpreted, the busulfan conditioning dose will default to a dose of 3.2 mg/kg by IV infusion over three hours once daily for two days. The 3.2 mg/kg dose will be calculated based on the recipient's ideal body weight or actual body weight, whichever is lower, except for recipients age < 16 where actual body weight will be used. For recipients who are greater than 120% of IBW, busulfan will be dosed on an adjusted IBW (IBW plus 25% of the difference between ideal and actual weight).

3.3.2 HCT Conditioning

HCT conditioning is the start of the research treatment phase of the study, beginning on day -14 for both arms.

Because the mechanism of action of pentostatin is adenosine deaminase inhibition and red blood cells contain high levels of adenosine deaminase, packed red blood cell (PRBC) transfusion should be avoided in recipients from the start of conditioning through day 0, unless necessary for bleeding or symptomatic anemia. If transfusion must be given during conditioning, it is preferred that the red cells be given at least 24 hours after pentostatin, if permitted by the clinical situation. Similarly, fresh frozen plasma transfusions should be avoided during conditioning unless clinically necessary.

Drugs that interact with busulfan should be kept as consistent as possible between the busulfan test dose and the conditioning doses of busulfan. If a recipient has significant changes in liver function and/or medications between the test dose and the conditioning doses, real-time busulfan kinetics can be used from the dose on day -3 to inform changes to the dose on day -2.

Actual body weight will be used for the dose calculations, except for the busulfan test dose and for PTCy and mesna, as noted below.

3.3.2.1 IOC Arm Conditioning Drugs

Agent	Dose	Days
e-ATG	40 mg/kg IV once daily	Transplant days -14 and -13
Prednisone (or methylprednisolone/ dexamethasone equivalent)	Tapering doses, given orally daily, and given prior to each daily dose of e-ATG on days -14 and -13 Days -14 through -12: 1 mg/kg/day Days -11 and -10: 0.75 mg/kg/day Days -9 and -8: 0.50 mg/kg/day Day -7: 0.25 mg/kg/day	Transplant days -14 through -7
Pentostatin	4 mg/m^2 IV infusion in 50-100 mL of 0.9% sodium chloride, once daily Pre-hydration (recipients ≥50 kg only: 1 liter of 0.9% normal saline over 120 minutes. Decisions regarding pre-hydration in recipients < 50 kg will be made on a clinical basis.	Transplant days -9 and -5
Cyclophosphamide[a]	5 mg/kg orally or IV once daily (dosage cap of 400 mg/day) Cyclophosphamide oral dose will need to be rounded to the nearest capsule increment (25 mg).	Transplant days -9 through -2

[a] Recipients will be advised to remain well hydrated (2-4 L of fluid intake per day) and to empty the bladder prior to sleeping while receiving oral cyclophosphamide.

3.3.2.2 RIC Arm Conditioning Drugs

Agent	Dose	Days
e-ATG	40 mg/kg IV once daily	Transplant days -14 and -13
Prednisone (or methylprednisolone/ dexamethasone equivalent)	Tapering doses, given orally daily, and given prior to each daily dose of e-ATG on days -14 and -13 Days -14 through -12: 1 mg/kg/day Days -11 and -10: 0.75 mg/kg/day Days -9 and -8: 0.50 mg/kg/day Day -7: 0.25 mg/kg/day	Transplant days -14 through -7
Pentostatin	4 mg/m^2 IV infusion in 50-100 mL of 0.9% sodium chloride, once daily Pre-hydration (recipients ≥ 50 kg only): 1 liter of 0.9% normal saline over 120 minutes. Decisions regarding pre-hydration in recipients < 50 kg will be made on a clinical basis.	Transplant days -11 and -7
Cyclophosphamide[a]	5 mg/kg orally or IV once daily (dosage cap of 400 mg/day) Cyclophosphamide oral dose will need to be rounded to the nearest capsule increment (25 mg).	Transplant days -11 through -4

Agent	Dose	Days
Busulfan	AUC targeted dose based on busulfan test dose or real time PKs, with a default dose of 3.2 mg/kg/day, given as IV infusion over 3 hours each day for 2 days	Transplant days -3 and -2

[a] Recipients will be advised to remain well hydrated (2-4 L of fluid intake per day) and to empty the bladder prior to sleeping while receiving oral cyclophosphamide.

3.3.3 GVHD Prophylaxis

GVHD prophylaxis will differ between the IOC and RIC arms with regard to PTCy dose. Please see GVHD Prophylaxis Table below for specific instructions.

It is crucial that no systemic immunosuppressive agents are given from day 0 until at least 24 hours after the completion of PTCy. This includes corticosteroids as anti-emetics. Hydrocortisone may be given for adrenal insufficiency during this time period, as necessary.

GVHD Prophylaxis Regimens

RIC arm recipients	Cyclophosphamide: 50 mg/kg IV once daily over 2 hours on days +3 and +4,[a] dosed according to ideal body weight[b] Mesna 50 mg/kg IBW as IV infusion concomitant with cyclophosphamide. Mesna is dosed according to ideal body weight, unless actual body weight is less.	Tacrolimus[d]: 0.02 mg/kg continuous IV infusion over 24 hours, starting on day +5, with dose adjustments to maintain a trough of 5-10 ng/mL, continued through day +90 with no taper. Doses should be modified as appropriate for drug interactions. Mycophenolate mofetil 15 mg/kg orally or IV three times daily (max 1000 mg/dose) starting on day +5, continued through day +25. Dosing will be according to actual body weight[c]

IOC arm recipients	Cyclophosphamide: 25 mg/kg IV once daily over 2 hours on days +3 and +4,[a] dosed according to ideal body weight[b] Mesna 25 mg/kg IBW as IV infusion concomitant with cyclophosphamide. Mesna is dosed according to ideal body weight, unless actual body weight is less.	Tacrolimus[d]: 0.02 mg/kg continuous IV infusion over 24 hours, starting on day +5, with dose adjustments to maintain a trough of 5-10 ng/mL, continued through day +90 with no taper. Doses should be modified as appropriate for drug interactions. Mycophenolate mofetil 15 mg/kg orally or IV three times daily (max 1000 mg/dose) starting on day +5, continued through day +25.

		Dosing will be according to actual body weight[c]

[a] Cyclophosphamide on day +3 should be given between 60-72 hours after the completion of stem cell infusion. On day +4, cyclophosphamide should be given approximately 24 hours after the dose on day +3. On the RIC arm, the dose may be reduced to 25 mg/kg/day on an individual patient basis, per the PI, if deemed to be at risk for toxicity from excessive chemotherapy, while also with disease status necessitating the RIC arm of the trial.

[b] Ideal body weight should be used to determine cyclophosphamide dose unless the recipient weighs less than ideal body weight, in which case actual body weight should be used.

[c] Mycophenolate mofetil may be continued beyond the protocol specified stop date if there is GVHD. Upon converting to oral mycophenolate mofetil, recipients less than 30 kg may require twice daily dosing instead of dosing three times per day to most closely approximate the target total daily dose given the limitations of pill denominations.

[d] Tacrolimus: when a recipient is reliably taking oral medications, tacrolimus will be converted to an equivalent oral dose using a 1:3 conversion from the IV form. The total daily dose will be divided into two equal doses, one dose given approximately every 12 hours. Tacrolimus may be continued beyond day +90 if there is GVHD requiring therapy, mixed chimerism, or other clinical indication.

3.3.4 Post-allo HCT Treatment with Filgrastim

G-CSF (filgrastim) may be given starting on day +5 at a dose of 5 mcg/kg/day (actual body weight) IV or subcutaneously, until the absolute neutrophil count is ≥ 1,000/mm^3 over the course of three days or ≥ 5,000/mm^3 on one day, although administration can be altered based on the patient's clinical situation. Rounding to the nearest vial is allowed. Additional G-CSF may be administered as warranted.

3.4 HCT GUIDELINES

On day 0, the recipient will receive fresh or cryopreserved stem cells from the donor. If cryopreserved, the stem cell product will be thawed and immediately administered intravenously. Target and minimum graft doses are listed in Section **3.2.** The PI will decide if greater than the target graft dose will be infused. The PI will also decide if additional collection can and should be done if the minimum graft dose is not reached. The graft will not be manipulated to deplete T cells. Processing for ABO incompatibility is as outlined in Section **3.2**. If the graft is cryopreserved, the recipient should be pre-medicated for DMSO-related toxicities (chills, muscle aches) with acetaminophen and diphenhydramine. If DMSO-related toxicities develop, the recipient may receive supportive care with additional diphenhydramine, merperidine, or other interventions as clinically appropriate.

3.5 DETERMINATION OF DONOR/HOST CHIMERISM AFTER HCT

All determinations of donor chimerism will be performed by the NIH Clinical Center pathology laboratory by PCR analysis of variable number of short tandem repeats. The initial baseline determination on donor and recipient will be performed by Clinical Center pathology prior to allo HCT. These assessments will be performed as outlined unless insufficient numbers of cells are present to perform the assays, or it is clinically unnecessary to perform the assay based on prior chimerism results. Unsorted donor chimerism studies on bone marrow will be performed at

day +60 and 1 year, and as clinically indicated. Lineage-specific chimerism studies will be performed by Clinical Center pathology after cells are sorted by the Blood Processing Core (BPC). Timepoints for chimerism assessment are outlined in the follow up guidelines and Study Calendar in Section **3.10**. Additional chimerism assessments should be performed as clinically indicated in recipients where there is a concern for decreasing donor chimerism and graft failure.

3.6 DONOR CELL INFUSIONS (DCI) AND SUBSEQUENT HCT

DCI includes $CD34^+$ selected-DCI or Lymphocyte-DCI, including CTL therapy. The use of immunosuppression/conditioning prior to DCI will be left to the discretion of the PI and will depend at least in part on if there is a suspected immune-basis behind the indication for DCI. Standard therapies directed at the underlying TCP/D disorder may be employed as a hopeful bridge to donor cell infusion or subsequent transplant on this protocol.

$CD34^+$ selected-DCI or virus-specific CTL therapies will not be performed as part of this protocol, although can be done for clinical need on other protocols or as standard of care while concurrently treated on this protocol. Recipients with viral complications due to EBV, CMV, or adenovirus pre- or post- allo HCT are permitted to concurrently enroll on virus-specific CTL clinical protocols to treat the viral infection and/or lymphoproliferative disorder. The CTLs may be either donor-derived or 3rd party products and would be collected and administered on another protocol. Any adverse event that occurs during co-participation in another clinical trial will be recorded and reported for this protocol to the IRB as outlined in Sections **6.1.1** and **7.2**.

For CIBMTR reporting, the terminology below, in concordance with established branch definitions regarding DCI vs subsequent allogeneic stem cell transplant, will be used.

When a recipient requires post-HCT therapy such as DCI or subsequent HCT, the recipient may or may not remain of the status “on protocol therapy”, depending on the type of therapy and whether or not the follow-up evaluations outlined by the study calendar remain relevant. For example, the follow-up evaluations largely center on immune reconstitution, phenotype reversal, and chimerism, which would not be relevant for a recipient with complete graft failure but likely would remain relevant in a recipient with mixed chimerism who is receiving a lymphocyte-DCI to improve the chimerism and potentially the immune reconstitution. For as long as the recipient remains of the status “on protocol therapy” (meaning following the protocol-specified evaluations post-HCT, even beyond day +90, when protocol-specified therapy ends), the study calendar should be followed, and the protocol-specified evaluations and specimens should be collected. When a recipient is taken off protocol therapy but remains on study, the study calendar and protocol-specified evaluations and specimen collection schedule no longer apply, and the follow-up should be guided by the recipient’s clinical status and necessary follow-up. However, the outcomes and immune recovery of recipients after graft failure or poor graft function who require DCI or subsequent HCT are of interest and relevant to the protocol’s secondary and exploratory objectives. Thus, data should continue to be recorded, as necessary to address objectives of the study.

3.6.1 Lymphocyte-DCI

Post-HCT lymphocyte-DCI is permitted for mixed/split donor chimerism (≤ 99% donor chimerism on whole blood, lymphoid, or myeloid subsets), for relapse/persistence of a pre-HCT hematopoietic malignancy, or for EBV-PTLD if virus-specific CTLs are not available. Use of lymphocyte-DCI prior to day +180 is permitted but discouraged as this carries a high risk of

GVHD. The tapering or discontinuation of immunosuppression should first be tried, if appropriate, to improve mixed/split donor chimerism, anti-tumor activity, or anti-viral immunity in cases of EBV lymphoproliferation. If clinically appropriate, rituximab should also first be tried as treatment for EBV-PTLD prior to pursuing lymphocyte-DCI, with failure of rituximab assessed at least one week after rituximab administration. Recipients should be without evidence of active, grade 2 or higher acute GVHD at the time of lymphocyte-DCI.

Lymphocyte-DCIs will be collected from the same donor used for the allo HCT, per DTM standard apheresis procedures. Lymphocyte-DCI will be given in a dose-escalation manner. Chemotherapy and/or immunosuppression may be given as part of the lymphocyte-DCI approach at the discretion of the PI. $CD3^+$ cell count will be determined by flow cytometry and will be used to calculate the lymphocyte-DCI dose. Additional CD3+ T cells will be cryopreserved for possible future use. Lymphocyte-DCI may be repeated approximately every four weeks with dose escalation as indicated, until therapeutic effect is achieved (improvement of donor chimerism, improvement of relapsed/persistent malignancy, or improvement of EBV-PTLD) or until GVHD develops or worsens from the pre-lymphocyte-DCI baseline. In addition to lymphocyte-DCI, recipients with relapsed/persistent malignancy may be offered therapy on other NCI protocols.

3.6.2 Autologous HCT Rescue

There may be recipients who have had autologous hematopoietic stem cells collected and cryopreserved off study. In cases of graft failure without autologous recovery or with insufficient autologous recovery (poor hematopoietic function), recipients who are not candidates for additional allogeneic cell therapy or who do not desire subsequent allogeneic cell therapy may receive their cryopreserved autologous hematopoietic stem cells as a potential rescue of hematopoietic function. The use of conditioning prior to autologous HCT will be at the discretion of the PI. However, autologous hematopoietic stem cells will not be collected for potential future use on this protocol.

3.6.3 Subsequent HCT

Given the presumed high risk for graft failure among recipients with these diseases of TCP/D, it is essential to have a mechanism to provide a potentially life-saving subsequent allogeneic HCT in the event of graft failure or falling/split donor chimerism that cannot be rescued with other methods. Thus, a subsequent allogeneic HCT may be given for the purposes of rescuing failing hematopoietic and/or immune function, or for salvaging a recipient with relapsing disease. The definition of an allogeneic HCT is the administration of any CD34+ cell containing product of at least 2 x 10^6 CD34+ cells/kg recipient ideal body weight. The administration of cells need not be preceded by preparative therapy but may at the discretion of the PI. The administration of cells need not be followed by GVHD prophylaxis but may at the discretion of the PI. If possible and time allows, chimerism in the blood or marrow should be assessed to determine that there are less than 5% donor myeloid cells present, although situations may arise where counts are too low for this assessment or the need for subsequent HCT is urgent and/or independent of the chimerism results. Donors for the subsequent allogeneic HCT may be the original donor or may be a new donor. Recipients will be followed after subsequent HCT from their new day Day 0, in accordance with CIBMTR reporting requirements.

3.7 TRANSPLANTATION FOLLOW-UP

3.7.1 Guidelines for timepoint and non-timepoint follow-up at NIH

Given the complexity of recipients post-HCT, unplanned clinical visits, consultations, evaluations, procedures, and testing may need to be performed in addition to the standard follow-up schedule outlined in the Study Calendar in Section **3.10**. A standard clinical order set (see **Appendix C**) will be used to facilitate ordering evaluations at both timepoint and non-timepoint follow-up visits.

3.7.2 Guidelines for follow-up outside of NIH

From day +100 through day +365, it is recommended that recipients be seen at least monthly by their local physician at home, if no longer being seen regularly at NIH. The timing and frequency will be determined by the PI in discussion with the local physician. The NIH study team will monitor the participants through review of medical notes from the home physician and the NIH team will communicate with the home physician as needed.

3.8 DOSE MODIFICATIONS

3.8.1 Anti-Thymocyte Globulin (e-ATG, Atgam)

Dose adjustments or modifications will not be made, unless there is a severe allergic reaction, in which case the infusion will be stopped and no further infusions will be given unless densensitization is possible as per the Allergy Consult Service, as per Section **4.6**.

3.8.2 Prednisone (For Serum Sickness Prophylaxis)

For recipients unable to take oral prednisone, methylprednisolone at 80% of the prednisone dose, or other equivalent steroid dose, such as dexamethasone, may be substituted. Prednisone or substitute may be continued beyond the scheduled serum sickness prophylaxis window outlined in the protocol (Sections **3.3.2.1** and **3.3.2.2**), as clinically appropriate and approved by the PI and/or LAI.

3.8.3 Cyclophosphamide (Cytoxan)

Low dose for conditioning regimen: Dosing will be rounded to the nearest capsule increment (25 mg). IV infusion in 1:1 dosing may be permitted if a recipient is unable to tolerate oral therapy.

High dose for GVHD prophylaxis: Cyclophosphamide is dosed according to ideal body weight, unless actual body weight is less. Recipients on the IOC arm, as well as select patients on the RIC arm, due to the potential genotoxic/clastogenic effect of cyclophosphamide, will receive PTCy at a reduced dose of 25 mg/kg/day on day +3 and +4.

3.8.4 Pentostatin (2-deoxycoformycin; Nipent®)

Pentostatin dose is calculated using the recipient's actual body weight. The dose of pentostatin will be 4 mg/m^2 if the recipient's pre-HCT creatinine is within the institutional normal range.

Organ Dysfunction: If the recipient's creatinine is not within the institutional normal range, a calculated creatinine clearance will be determined pre-HCT and before the second dose of pentostatin. If the calculated creatinine clearance is between 40 and 59 ml/min, the dose of pentostatin will be 3 mg/m^2. If the calculated creatinine clearance is between 30-39 ml/min, the dose of pentostatin will be 2 mg/m^2. If the calculated creatinine clearance falls below 30 ml/min,

further pentostatin doses will be held. If a recipient develops evidence of renal insufficiency during the conditioning regimen, as defined by an increase in serum creatinine of more than 33%, then the estimated creatinine clearance (based on urine collection (at least 4-hour) on the day of the second pentostatin dose should be used for calculating that dose.

Toxicity: Because pentostatin is rarely associated with neurotoxicity (seizures, ataxia, encephalitis), special attention should be paid towards evaluating central nervous system toxicity. In the event that the treatment is associated with any neurologic toxicity of grade 2 severity or higher, the PI should be contacted to discuss whether further pentostatin therapy is advised. Severe rash potentially attributable to pentostatin should also be discussed with the PI to determine if further therapy is advised.

3.8.5 Busulfan

Busulfan dose will be determined as outlined in Section **3.3.1**. As outlined in that section, adjustments can be made to the second dose of busulfan based on real-time pharmacokinetic data collected during the first dose, if there is a concern that the recipient's clearance has changed significantly from his/her clearance during the test dose.

3.8.6 Mesna

Dose adjustments or modifications will not be made.

3.8.7 Mycophenolate Mofetil

Drug Interactions: MMF activity is decreased with oral antacids and cholestyramine. Acyclovir or ganciclovir blood levels may increase due to competition for tubular secretion. High doses of salicylates or other highly protein-bound drugs may increase the free fraction of mycophenolic acid and exaggerate the potential for myelosuppression.

Organ Dysfunction: No dose adjustments are required for liver dysfunction. For renal insufficiency, MMF dosing should not be modified unless dialysis is needed, in which case MMF can be reduced to 25-50% of the starting dose.

3.8.8 Tacrolimus

Therapeutic Targets: The tacrolimus dose should be adjusted to maintain a serum trough level of 5-10 ng/mL. Changes in levels due to altered bioavailability should be apparent within 24-48 hours. A 20-25% dose reduction is recommended for trough levels of 10-16 ng/mL, and a 20-25% increase is recommended for trough levels < 5 ng/mL.

Organ Dysfunction: Renal failure does not affect the excretion of tacrolimus. Excretion is reduced in liver failure; impaired hepatic function should prompt consideration of reduction in tacrolimus dose.

Drug Interactions: Due to extreme interactions with voriconazole and posaconazole, these drugs are relatively contraindicated during tacrolimus therapy. Tacrolimus dose should be reduced when or shortly after azoles are initiated. All dose adjustments should be made in consultation with the pharmacy.

Toxicity: If tacrolimus toxicity develops that is significant, such as microangiopathy, the drug may be stopped, changed to an alternative immunosuppressant drug (such as sirolimus or cyclosporine), or dose-reduced, as clinically appropriate based on the individual situation.

Reversal of azole-mediated inhibition of cytochrome CYP3A4 and P-glycoprotein is gradual. Therefore, immediate significant dose increases are not advised. Rather tacrolimus dose increases should be cautious and based on more frequent monitoring of levels as appropriate.

3.9 USE OF EXISTING DATA

For participants who are co-enrolled on 17-I-0122 and this study (18-C-0135), results of genetic sequencing performed on 17-I-0122 may be used with participant permission to better understand a recipient's underlying disease process, treatment options, and risk factors for complications on a clinical basis. If a pathogenic germline mutation is identified in a recipient prior to transplant, this information may be used to exclude related donors who are affected by the same mutation, again on a clinical basis. Identifiable data obtained on 18-C-0135 and shared with the 17-I-0122 research team may include patient history and results of clinical evaluations to provide context of interpretation of genetic sequencing results. Rarely, pre-HCT bone marrow and blood specimens that have already been previously collected (Section **5**) may be shared for retrospective genetic evaluation if a patient is co-enrolled on 17-I-0122 and has given permission for the sharing of identifiable data and specimens.

3.10 STUDY CALENDAR[Q]

Evaluations (Recipient)	Screen		Baseline Evals	Start of conditioning through day 0	Day +1 through day+27	Day +28 (± 2 days)	Day +42 (± 3 days)	Day +60 (± 3 days)	Every 7 ± 3 days from day +29 through day +99	Day +100 (± 3 days)[r]	Day +180 (± 14 days)[r]	Day +365 (± 21 days)[r]	Day +548 (18 months) (± 28 days)[r]	At +2 years and yearly thereafter through +5 years (± 56 days)[r]
	Any time pre rx	≤ -4 wks pre rx												
Vital signs, weight, performance Status assessment[a]		X	X			X	X	X		X	X	X	X	X
Path review[c], evaluation of central venous access potential[k], ABO, HLA typing, HLA ab screen,	X[k]													
Complete History, Exam, HIV-1/2 antibody screen and quantitative HIV-1 RNA PCR, current medications		X												
HSCT Note: interim hx, focused PE, post-HCT GVHD eval[b]				X[h]	X[h]	X	X	X	X	X	X	X	X	X
Antibody screens for HSV I/II, VZV, West Nile Virus, HTLVI/II, Hepatitis A, Hepatitis B, Hepatitis C, Toxo, T cruzi; syphilis screen; QuantiFERON; Peripheral blood smear, G6PD screen, Hgb electrophoresis, fibrinogen, isohemagglutinin (if applicable); STR Profile; EKG; Dental, Nutrition, Transplant ID, Social Work Consults; HCT-CI calculation; Concomitant medications			X											
Acute care/hepatic/mineral panels		X		X[h]	X[h]	X	X	X	X	X	X	X	X	X
CBC with diff, reticulocyte count			X	X[h]	X[h]	X	X	X	X	X	X	X	X	X
Cytokine panel, ESR, CRP, ferritin			X	X[j]	X[j]	X	X	X		X	X	X	X	X
LDH, uric acid, PT, PTT, total protein; gamma glutamyl transferase; Quantitative blood PCRs for EBV, CMV, adenovirus, BK virus, JC virus, and HHV6; qualitative blood PCR for toxoplasma; quantitative PCR for BK and JC virus in the urine; qualitative PCR for adenovirus in the urine; lymphocyte phenotyping – TBNK[t]; Urinalysis with microscopic evaluation, spot urine protein creatinine ratio			X	X[i]	X[i]	X	X	X	X	X	X	X	X	X
Quant Igs (IgG, IgA, IgM), molecular TRG/IGH, fasting lipids, iron and transferrin panel			X			X	X	X		X	X	X	X	X
IgE, 25-hydroxyvitamin D, 1,25-hydroxyvitamin D			X							X	X	X	X	X
Serum or urine β-HCG (females of childbearing potential)		X	X											
Donor chimerism in blood (WBC or WB; lineage-specific[d])					X[m]	X	X	X	X[m]	X	X	X	X	X
PFTs, 2D ECHO, height		X[n]									X[n]	X[n]	X[n]	X[n]
CMV and EBV serology	X[k]											X		X

Evaluations (Recipient)	Screen		Baseline Evals	Start of conditioning through day 0	Day +1 through day+27	Day +28 (± 2 days)	Day +42 (± 3 days)	Day +60 (± 3 days)	Every 7 ± 3 days from day +29 through day +99	Day +100 (± 3 days)[r]	Day +180 (± 14 days)[r]	Day +365 (± 21 days)[r]	Day +548 (18 months) (± 28 days)[r]	At +2 years and yearly thereafter through +5 years (± 56 days)[r]
	Any time pre rx	≤ -4 wks pre rx												
Bone marrow aspirate and biopsy[e]		X						X				X		
Research Blood[f]			X	X	X[l]	X	X	X		X	X	X	X	X
Vaccinations[g]											X	X		X
Endocrinology labs[s] ± consult, DEXA[o]			X									X		X
Gynecology evaluation for GVHD, cancer screening												X[p]		X[p]

a Per **Appendix A**

b Per **Appendix B**.

c For recipients with a history of malignancy, lymphoproliferative disorder, HLH, LGL, or other disease manifestations captured on tissue biopsy, available diagnostic tissue specimens should be sent for NIH pathology review, as clinically indicated

d Specimens for lineage-specific chimerism will first be sorted by the Blood Processing Core (BPC), then sent to DLM for chimerism studies

e With flow cytometry, as well as IHC, molecular testing, and cytogenetics/FISH as appropriate; chimerism if post-allo HCT; pre-HCT bone marrow aspirate and biopsy may need to be performed to solidify eligibility, or may not need to be performed until baseline evaluations (not essential for eligibility/screening). Bone marrow biopsy may need to be repeated at baseline if interim therapy is administered after screening, per PI discretion.

f Per table in Section **5.1** Pre-HCT research specimens should be timed for collection in a way that is appropriate based on the recipient's pre-HCT therapies, accounting for chemotherapy or other treatments, count recovery after therapy, etc.

g Per guidelines at: https://cccasper.cc.nih.gov/cvpn/aHR0cDovL2ludHJhbmV0LmNjLm5paC5nb3Y/bmt/clinicalcare/pdf/Table_II.pdf; vaccination schedule may be delayed if recipient develops GVHD or other complication; vaccinations scheduled to be performed between months 14-18 post-allo HCT should be arranged to be done through the recipient's home transplant physician; day +180 vaccinations should include the Pneumococcal 13-valent conjugate vaccine for all recipients and the seasonal influenza vaccine if available. Antibody titers for hepatitis B virus, measles, tetanus, diphtheria, polio, and Streptococcus pneumoniae will be measured to determine response to vaccinations once completed.

h Performed daily, unless not hospitalized or deemed unnecessary for clinical management. If not hospitalized, once weekly evaluations are sufficient.

i Performed weekly (± 2 days), preferably timed so that one evaluation time point falls on day 0, except CMV/EBV PCR which should be performed twice weekly during conditioning

j Only perform the following labs at the following timepoints: Cytokine panel, ferritin, ESR, and CRP on days -14, -7, and 0. Cytokine panels to be collected on day +3, +5, +7, +14, and +21; CRP performed daily from day +1 through day +21

k Evaluations may be performed at any time prior to study entry and may not need to be repeated at screening or baseline if result is not expected to change, such as blood type, positive EBV and/or CMV serostatus, etc. HLA typing will need to be repeated within 2 years of HCT, as outlined in Section **2.2.2**.

l Research samples scheduled for collection on day +14 may be collected on day +14 ± 1 day. Research samples scheduled to be collected on days +1 through +7 must be collected on that day, with particular attention to timing of specimen collection relative to clinical interventions (administration of PTCy, G-CSF, etc.). See table in Section **5.1** for further timing details

m WBC STR Chimerism or whole blood chimerism - Specimen should be sent when post-nadir WBC count is > 200 cells/µL, around day +21, as well as day +35 ± 3 days. Lineage specific chimerism will not be sent routinely on day +21 or day +35.

n PFTs should include both pre- and post-bronchodilator evaluation, if clinically indicated.

[o] DEXA only as clinically needed and, after HCT, every two years, unless more frequent monitoring clinically necessary; this surveillance is highly recommended but not a deviation if not performed.

[p] Age-appropriate female recipients only; gynecology evaluation may be performed at home in lieu of evaluation at NIH; post-transplant gynecology follow up is highly recommended but not required

[q] The recording of adverse events is outlined in Section **6.1.1,** as the events recorded vary by timepoint and recipient status on the study.

[r] Follow-up visits may be completed by remote visit with a member of the study team (e.g., if the participant is not able to return to the NIH CC). Remote visits will be conducted in compliance with NIH guidelines and FDA regulations. A participant may be referred to their local provider or asked to come to the NIH CC for an in-person assessment, if clinically indicated, and at the discretion of the investigator. In the case of any visits with participants' local providers, records will be obtained for the research records.

[s] For post-pubertal recipients: thyroid stimulating hormone (TSH), free T4, total T3, adrenocorticotropic hormone (ACTH), morning cortisol, parathyroid hormone (PTH), males only- morning testosterone (free and total); hemoglobin A1c, fasting glucose, follicle stimulating hormone (FSH), luteinizing hormone (LH), females only – estradiol. Pre-pubertal recipients will have age appropriate labs drawn as recommended by pediatric endocrinology.

[t] More extensive lymphocyte phenotyping panel orders that include TBNK (such as Lymphocyte Phenotyping – PID panel) may be substituted as clinically appropriate.

3.11 Cost and Compensation

3.11.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs.

3.11.2 Compensation

Participants will not be compensated on this study.

3.11.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.12 Criteria for Removal from Protocol Therapy and Off Study Criteria

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days from the last dose of study therapy. The last dose of study therapy will be tacrolimus on day +90. Early removal from study is expected to be quite unlikely, as most recipients will continue protocol follow-up on study through 5 years post-HCT, long after protocol therapy has ended.

For subjects who are removed from protocol therapy but who remain on study: Upon removal from protocol therapy, protocol-specified follow-up evaluations and documentation per the study calendar are not required, as the recipient will cease to be on protocol therapy and its associated follow-up. After removal from protocol therapy, unanticipated problems and grade 5 adverse events will be recorded and reported. In addition, adverse events occurring in off protocol therapy subjects that are relevant to the objectives of the study will continue to be recorded and relevant laboratory tests and evaluations will continue to be loaded into the study database, for the purposes of data collection and addressing objectives of the study. However, the adverse events occurring in off protocol therapy subjects, except for grade 5 adverse events and unanticipated problems, will not be reported to the IRB. Recipients who are taken off protocol therapy but who remain on study to receive additional necessary standard therapies, including subsequent cell infusion, may be treated per standard of care on this study.

A flow diagram of subject status, from screening through off study, is shown in **Appendix D**.

3.12.1 Criteria for removal from protocol therapy

- Participants request to be withdrawn from active therapy
- Investigator discretion
- Unacceptable toxicity
- Participant becomes pregnant
- Recipient: Failure of protocol therapy, necessitating ongoing non-protocol defined standard therapies, including some (but not all) recipients requiring subsequent cell infusion.

The PI is to be notified of all discontinuations of study drugs. The reason for dose modifications (except for routine adjustments to tacrolimus doses to maintain a target trough)/discontinuation should be recorded in the CRF and in the recipient's medical records.

3.12.2 Off-Study Criteria

- Donors: upon recipient removal from study, unless adverse events are still being followed in the donor despite recipient being taken off study.
- Participant requests to be withdrawn from the study
- Potential recipients for whom a suitable donor cannot be found
- Failure to meet pre-HCT eligibility criteria as defined in Sections **2.1.1** and **2.1.2** (Recipient) and sections **2.1.3** and **2.1.4** (Donor)
- Subject lost to follow-up
- Death
- Completed follow up period
- PI decision to end this study
- Adult donor subjects who become decisionally impaired
- Screen Failure

3.12.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the visit window and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

Note: The below are guidelines and suggested medications and schedule to be used; however, they can be altered by the treating physician as clinically indicated.

4.1 SUPPORTIVE CARE

HCT is a long process that, even without complications, requires extensive supportive care, evaluations, monitoring, and interventions that are standard to the field of HCT and are too myriad to detail fully here. As part of promoting optimal post-HCT survivorship, care that is

standard to the support of the post-HCT recipient is necessary and may be provided on this protocol. There are many potential complications that require standard therapies and interventions post-HCT, including disease relapse, graft failure or poor graft function, infection, GVHD, SOS, idiopathic pneumonia syndrome, delays in immune reconstitution, and so forth.

4.2 Central Venous Access

Adequate central intravenous access with a central venous catheter is required for all allo HCT recipients on this trial for administration of IV medications and blood products. A non-tunneled, staggered triple lumen catheter is preferred. Implanted ports are not sufficient central venous access for allo HCT, but ports may remain in place to allow for the earlier removal of the temporary second central venous line.

Catheters should be removed as soon as possible after hematopoietic recovery, unless the clinical situation dictates that the line remain in place.

4.3 Seizure Prophylaxis

Busulfan is known to cross the blood-brain barrier and can induce seizures. Recipients on the RIC arm will be pre-medicated with clonazepam plus levetiracetam for prophylaxis against seizures. For adults, clonazepam 0.5 mg orally every 12 hours and levetiracetam 500 mg orally every 12 hours will be started the evening prior to busulfan and continue until the morning following the last dose of busulfan. For pediatric recipients, the levetiracetam dose will be 10 mg/kg (maximum dose 500 mg) orally (preferred) or IV every 12 hours. Levetiracetam oral doses may be rounded to accommodate tablet sizes. The clonazepam dose for pediatric recipients who are 10 years or older OR ≥ 30 kg will be equal to the adult dosing. Pediatric recipients less than 10 years old OR < 30 kg will receive clonazepam 0.005-0.015 mg/kg orally every 12 hours, with doses rounded to available tablet sizes. Alternative prophylactic regimens may be used at the discretion of the PI. Anti-seizure prophylaxis is not required for the busulfan test dose.

4.4 Infection Prophylaxis

Allo HCT recipients will receive infection prophylaxis and treatment according to NIH BMT Consortium Supportive Care Guidelines at:
http://intranet.cc.nih.gov/bmt/clinicalcare/infectionmanagement.shtml.

4.5 Serum Sickness

E-ATG can cause serum sickness, manifested as flu-like symptoms. Recipients will receive systemic corticosteroids as outlined in Section **3.8.2**. The use of systemic corticosteroids for serum sickness beyond day -7 will be decided based on clinical indication in consultation with the PI or LAI.

4.6 E-ATG Infusion Reactions

Infusion reactions will be treated symptomatically as clinically indicated and treatments may include antiemetics, IV fluids, acetaminophen, antihistamines, inhaled bronchodilators, merperidine, or other clinically appropriate interventions. In the case of moderate or severe reactions, hydrocortisone may be given, and the infusion will be discontinued and restarted at a slower rate once the symptoms have subsided. If a recipient has a persistent severe infusion reaction that does not respond to measures to ameliorate the signs and symptoms associated with the infusion, the infusion will be discontinued, and further study participation will continue as

planned with the exception that no further e-ATG will be administered, unless desensitization is recommended by the Allergy Consult Service.

Platelets should be supported during and after e-ATG infusion as outlined in Section **3.8.1**.

4.7 GROWTH FACTORS

G-CSF (filgrastim) may begin on day +5, as clinically indicated, at a dose of 5 mcg/kg/day (actual body weight) and is administered daily subcutaneously or IV until the absolute neutrophil count is ≥ 1000 cells/mm^3 for three days or ≥ 5000 for one day. Rounding to the nearest vial is allowed. Additional G-CSF may be administered as clinically warranted or may be stopped early. For recipients with absolute neutrophil count ≥ 1000 cells/mm^3 on day +5, G-CSF can be held until counts begin to nadir at the discretion of the PI. Pegfilgrastim and GM-CSF are not permitted.

4.8 ANTI-EMETICS

Note that steroids should not be used as an anti-emetic agent after the graft is infused on day 0. If the event of ongoing serum sickness, efforts should be made to stop or limit steroid exposure from days 0 through +4. Steroids should, if at all possible, not be given until at least 24 hours after the completion of all PTCy. Antiemetic usage will otherwise follow Clinical Center guidelines (https://cccasper.cc.nih.gov/cvpn/aHR0cDovL2ludHJhbmV0LmNjLm5paC5nb3Y/pharm/pdf/NIHCCAntiemeticGuidelinesSeptember2011FinalA.pdf) and recommendations from the Pharmacy.

4.9 BLOOD PRODUCT SUPPORT

Recipient's blood counts will be monitored at least daily during hospitalization for allo HCT. Recipients will receive PRBCs and platelets as needed to maintain a hemoglobin > 8.0 g/dL and platelets > 10,000/mm3 (or higher if clinically indicated), unless the recipient is not group O blood type and the donor is, in which case the hemoglobin will be kept > 9.4 g/dL day +4 through day +14 per NCI practice. **From the start of pentostatin through day 0, recipients should not receive PRBC transfusion unless absolutely necessary for bleeding or symptomatic anemia given the high levels of adenosine deaminase in red blood cells and the potential for PRBC transfusion to rescue the adenosine deaminase inhibition induced by pentostatin. If PRBC transfusion must be given during conditioning, it is preferred that the red cells be given at least 24 hours after pentostatin, as permitted by the clinical situation. Similarly, FFP transfusions should be avoided during conditioning unless clinically necessary.**

Management of ABO incompatible donor cell infusions is further outlined at https://cccasper.cc.nih.gov/cvpn/aHR0cDovL2ludHJhbmV0LmNjLm5paC5nb3Y/bmt/clinicalcare/pdf/ABO.pdf.

Recipients with fever and thrombocytopenia should be transfused to keep platelets > 20,000/mm^3 while febrile. During e-ATG infusion through day -10 at minimum, platelets should be kept greater than 20,000/mm^3 given the transient thrombocytopenia that may occur with e-ATG.

All blood products, with the exception of the stem cell products and DCI, will be irradiated and leukoreduced. CMV seronegative recipients with CMV seronegative donors should receive

CMV-negative blood products whenever possible. Blood product support as outlined above will begin at a minimum of two weeks prior to day 0 and continue for at least one year after allo HCT. Recipients receiving immunosuppressive medications will continue to have all blood products irradiated until discontinuation of immunosuppression.

Isohemagglutinin titers will be checked on recipients prior to HCT (unless blood type AB), as well as serially post-HCT as clinically indicated in the setting of major ABO mismatch and as outlined in the study calendar (Section **3.10**).

4.10 Minimizing Bleeding Risk in Recipients with MAGT1 Deficiency (XMEN)

We think that there may be increased risk of bleeding during post-HCT aplasia in recipients with MAGT1 deficiency (XMEN). The mechanism of increased bleeding risk is not understood, and, at present, the perceived increased risk of bleeding is based on observations by the PI and other members of the protocol team, in addition to personal communications with outside providers who have transplanted patients with MAGT1 deficiency. The bleeding risk seems to be predominantly mucosal, suggesting a platelet disorder. Thus, all patients with *MAGT1* deficiency on this protocol will undergo pre-HCT ENT evaluation, imaging of the nose/sinuses/base of skull, as well as laboratory evaluation of platelet number and function, as well as coagulation, as appropriate and possible. Some platelet function studies may not be possible, as a platelet count of > 100,000/uL is required to do studies such as platelet aggregation. Additionally, DTM will be consulted on *MAGT1* patients prior to HCT to formulate a plan as how to best support the patient with platelet transfusions during aplasia. A platelet goal of 30,000/uL will be targeted for these patients, although may not be obtainable in all patients. Patients with *MAGT1* deficiency will not be eligible if they require anti-platelet and/or anti-coagulation therapies that cannot be interrupted during HCT. *MAGT1* patients should not be given anti-platelet agents or anti-coagulation while on study unless approved by the PI.

4.11 Immunoglobulin Replacement Therapy

Immunoglobulin replacement therapy (IV or subcutaneous) may be used before and/or after HCT to maintain adequate IgG levels as appropriate for the prevention of infections. Immunoglobulin replacement therapy should not be administered during conditioning, if possible. The administration of immunoglobulin replacement should be avoided through one week after allo HCT due to the potential increased risk of SOS.

4.12 Hepatic Function Support

All recipients will receive ursodeoxycholic acid for the prevention of hepatic complications after allo HCT, unless deemed to not be clinically indicated or advisable by the Investigator.(95) Ursodiol will start on the first day of conditioning, or sooner if clinically indicated, and will continue until day +100, or longer if clinically indicated. Recipients weighing less than 90 kg will receive 300 mg orally twice daily. Additional dose reductions for recipients weighing less than 40 kg should be discussed with the pharmacy. Those weighing more than 90 kg will receive 300 mg orally each morning and 600 mg orally each evening.

4.13 Prevention of Hemorrhagic Cystitis

Hemorrhagic cystitis is a well-recognized potential complication of high-dose cyclophosphamide therapy. The approach to the prevention of hemorrhagic cystitis is as outlined in the NIH BMT

Consortium Supportive Care Guidelines at:
http://intranet.cc.nih.gov/bmt/clinicalcare/pdf/HemCystitis_Prevent_guideline_2007.pdf.

Hemorrhagic cystitis should be graded according to the following (Droller criteria):(96)
Grade 1: Microscopic hematuria
Grade 2: Macroscopic hematuria
Grade 3: Macroscopic hematuria with clots
Grade 4: Life threatening macroscopic hematuria with clots leading to renal failure or urinary tract obstruction requiring instrumentation for removal

4.14 Anti-ovulatory Treatment

Menstruating females should begin an anti-ovulatory agent before starting the conditioning regimen, or be managed per specific recommendations by Reproductive Endocrinology and Infertility specialists if such specialists are involved in the care of the subject prior to allo HCT, such as for egg harvesting and preservation. A full description of the recommended approach to menses suppression is available in the NIH BMT Consortium Supportive Care Guidelines at: http://intranet.cc.nih.gov/bmt/clinicalcare/pdf/menses_suppression.pdf.

4.15 Engraftment Syndrome

Engraftment syndrome may occur around the time of neutrophil recovery. Its clinical manifestations include fever, rash, and vascular leak causing non-cardiogenic pulmonary edema, weight gain, and renal insufficiency. Diagnostic Spitzer criteria are as follows:

Major criteria	• Fever > 38.3°F with no identifiable infectious etiology • Erythrodermatous rash involving more than 25% of the body surface area and not attributable to medication • Noncardiogenic pulmonary edema with diffuse pulmonary infiltrates and hypoxia
Minor criteria	• Hepatic dysfunction: either total bilirubin > 2 mg/dL or transaminases > 2x ULN • Renal insufficiency (serum creatinine > 2 times baseline) • Weight gain > 2.5% of baseline body weight • Transient encephalopathy unexplainable by other causes

A diagnosis of engraftment syndrome is established by the presence of all three major criteria, or two major criteria and one or more minor criteria. The clinical signs and symptoms should appear within ± 4 days of neutrophil recovery.

4.16 Assessment of GVHD Response at 7 Days

1) Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD
2) Partial response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of performance status.
3) Non-responder: < 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Failure to maintain performance status.
4) Progressive disease: Further progressive signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

The signs and symptoms of chronic GVHD are provided in **Appendix B**. An assessment tool and chronic GVHD Score Sheet is provided in **Appendix B**. Chronic GVHD assessments and scoring should be performed at diagnosis, as well as at follow-up on days +60, +100, +180, 1 year, and yearly thereafter through +5 years after allo HCT, at minimum.

4.17 IMMUNIZATION POST-HCT

Allo HCT recipients will be immunized according to the BMT Consortium guidelines, as outlined in the table, available at: http://intranet.cc.nih.gov/bmt/_pdf/table_II.pdf.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH

1) Fluorescence-activated cell sorting (FACS) immune evaluation: Immunophenotyping of immune cell subsets by multicolor flow cytometry prior to HCT, during conditioning and post-HCT immune reconstitution. These studies will be done to gain insight into the dynamics of immune reconstitution after allo HCT using PTCy and distal e-ATG. These studies will be performed in blood and marrow on all participants, as well as in other body fluids (pleural fluid, ascites, CSF, urine, stool, etc) when of potential research interest.

2) T-, B-, and NK cell profiling (TBNK): TBNK profiling by multicolor flow cytometry will be important for assessing recipients post-HCT. The BPC in collaboration with the Laboratory of Pathology has the ability to obtain TBNK data from specimens containing fewer cells than the lower limit for the clinical core lab. Thus, specimens for TBNK profiling will be collected and evaluated by the BPC in cases where there are insufficient cells to run the flow cytometry in the core laboratory.

3) Cytokines/Proteomics: There are several promising soluble biomarkers of diagnostic and prognostic importance in GVHD. Similarly, other inflammatory states that can occur post-HCT, such as severe infection, viral reactivation, autoinflammation, immune dysregulation, graft failure, etc. may be associated with changes in the cytokine/chemokine profiles in cell-free blood specimens. These soluble markers will be quantified by ELISA in subject samples and compared across subjects, grouped by the occurrence or absence of clinical events such as those listed above.

4) FACS, sequencing, intracellular markers: These flow cytometry and PCR-based studies will allow further characterization of the dynamics of immune reconstitution after allo HCT. T-cell receptor sequencing of T-cell-receptor genes/gene segments of the alpha and beta chains will use next generation sequencing. The T-cell receptor sequencing studies will evaluate recombination events in response to pathogens and will inform analyses of antigen specificity after allo HCT and the recovery of virus-specific immunity. Given that sequencing will only involve the dynamic recombination events of the T-cell receptor to characterize repertoire diversity and virus-specific cellular immune response after transplant, incidental findings and their management are not a concern. The sequencing that will be done on the T-cell receptor will not reflect germline changes and have no clinical significance for the subjects, as these are dynamic recombination events in response to pathogen exposure. These studies will be performed in blood and marrow on

all participants, as well as in other body fluids (pleural fluid, ascites, CSF, urine, stool, etc) when of potential research interest.

5) Lineage-specific chimerism: Recipient blood specimens will be flow sorted into fractions of CD19 (B), CD4 (T), CD8 (T), CD56 (NK), and CD14 (monocyte) cells, followed by chimerism analysis to evaluate post-allo HCT chimerism and lineage-specific engraftment, as well as assess for split chimerism.
6) Mycobacteria-related and/or CMV-related immune reconstitution inflammatory syndrome (IRIS) after allo HCT: In appropriate recipients, blood will be collected from recipients with mycobacterial infections and/or CMV infections pre- and post-HCT for studies to evaluate their native and transplanted immune system's response to mycobacterial and CMV antigens through cellular functional assays. This will be in collaboration with Dr. Irini Sereti's lab.
7) Activity of e-ATG: Flow based studies evaluating binding of e-ATG against different cell lineages will be performed in Dr. Chris Kanakry's lab .

A schedule of biospecimen collections from recipients and donors for correlative studies is outlined in the tables below. The amount of blood that may be drawn from adult recipients and donors (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. For pediatric recipients and donors, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. In the event that blood draws are limited due to subject size, research studies will be performed in order of priority, per PI discretion. Although suggested volumes of blood for each research assay are outlined in the table below, these are estimated necessary volumes based on predicted blood counts. Therefore, smaller volumes of blood may be collected for each assay, per PI discretion, so as to minimize excess phlebotomy when possible, both based on the clinical appropriateness for the subject and the subject's blood counts and projected yield of cells from a given volume.

Bodily fluids such as ascites, pleural fluid, CSF, urine, stool, etc may be collected for the studies listed in 1 and 4 above. For fluids that require invasive procedure to obtain, these fluids will be collected and stored for research studies only at times of a procedure for clinical purposes. No spinal taps, paracenteses, or thoracenteses will be performed solely for research purposes. No more than 5 mL of CSF will be taken for research studies at the time of a clinical spinal tap.

The sampling time points are outlined in the table. For Pre-HCT specimens, the timing of these specimens in relation to pre-HCT therapies will be determined by the PI, as the optimal timing may differ from subject to subject. However, all pre-HCT specimens should be drawn prior to day -14 and ideally prior to the busulfan test dose, although that is not required. Additional research blood, marrow, and other bodily fluids (within the above parameters) may be collected at any time during the clinical course of on study subjects (either on or off protocol therapy), at the discretion of the PI, within the volume restriction limits, allowing for research studies related to secondary and exploratory objectives to be performed at the time of an unanticipated clinical event. The tables specify whether specimens will be batched and performed retrospectively or performed in real time for each assay. Cell lines will not be created.

5.1.1 Recipient Scheduled of Biospecimen Collection for Correlative Studies

Table 1

Day/Time point	Spec Type	Correlative Study	Volume[a]	Location of initial processing/specimen analysis
Day -12	PB	ATG binding and levels	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC→ Chris Kanakry lab
Day -10	PB	ATG binding and levels	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC→ Chris Kanakry lab
Day -8	PB	ATG binding and levels	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC→ Chris Kanakry lab
Day -7	PB	ATG binding and levels	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC→ Chris Kanakry lab
Day -4	PB	ATG binding and levels	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC→ Chris Kanakry lab
Day -3	PB	ATG binding and levels	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC→ Chris Kanakry lab
Pre-HCT	PB*	FACS Immune eval	4 panels (up to 6-8 mL red/green CPT tubes)	BPC
Pre-HCT	PB*,%	TBNK	1-3 mL lavender tube	BPC
Pre-HCT	BM*	FACS Immune eval	4 panels (1-10 mL green top tube, viably freeze remainder)	BPC
Pre-HCT	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Pre-HCT	PB	FACS, seq, intracell markers	Viably frozen PBMCs: 5 x 10^6 aliquots (up to 6-8 mL CPT)	BPC
Pre-HCT	BM	FACS, seq, intracell markers	Viably frozen cells: 10 x 10^6 aliquots (1-10 mL green top tubes)	BPC
Pre-HCT	BM*	ATG binding and levels	From green top tube for FACS	BPC→ Chris Kanakry lab
Pre-HCT	PB*	ATG binding and levels	From red/green CPT tube for FACS	BPC→ Chris Kanakry lab
Pre-HCT^	PB&	MAC-IRIS (Sereti)	Up to 3-10 mL green top tubes	BPC
Pre-HCT^	PB&	MAC-IRIS (Sereti)	6 mL EDTA tube for plasma storage (1 mL aliquots)	BPC
Day 0	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day 0, prior to stem cell infusion	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC
Day +1	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC
Day +2	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC
Day +3	PB*	FACS Immune eval	4 panels (up to 6-8 mL red/green CPT tubes)	BPC
Day +3, prior to PTCy infusion #1	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +4, prior to PTCy infusion #2	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC
Day +5, prior to immunosuppression & G-CSF	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC
Day +6	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (1-10 mL green top)	BPC
Day +7	PB*	FACS Immune eval	4 panels (up to 6-8 mL red/green CPT tubes)	BPC
Day +7	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +7	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +14	PB*	FACS Immune eval	4 panels (up to 6-8 mL red/green CPT tubes)	BPC

Day/Time point	Spec Type	Correlative Study	Volume[a]	Location of initial processing/specimen analysis
Day +14	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +14	PB	FACS, seq, intracell markers	Viably frozen PBMC: 5 x 10^6 aliquots (up to 5-8 mL CPT)	BPC
Day +14	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +28	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +28	PB*	Lineage specific chimerism	Up to 6-8 mL red/green CPT tubes	BPC→DLM
Day +28	PB*	FACS Immune eval	4 panels (up to 6-8 mL red/green CPT tubes)	BPC
Day +28	PB	FACS, seq, intracell markers	Viably frozen PBMC: 5 x 10^6 aliquots (up to 5-8 mL CPT)	BPC
Day +28	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +42	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +42	PB*	Lineage specific chimerism	Up to 6-8 mL red/green CPT tubes	BPC→DLM
Day +60	PB*	FACS Immune eval	Enough for 4 panels (up to 6-8 mL red/green CPT tubes)	BPC
Day +60	PB*	Lineage specific chimerism	6-8 mL red/green CPT tubes	BPC→DLM
Day +60	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +60	PB	FACS, seq, intracell markers	Viably frozen PBMC: 5 x 10^6 aliquots (up to 5-8 mL CPT)	BPC
Day +60	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +60	BM*	FACS Immune eval	4 panels (1-10 mL green top tube, viably freeze remainder)	BPC
Day +60	BM	FACS, seq, intracell markers	Viably frozen cells: 10 x 10^6 aliquots (1-10 mL green top tubes)	BPC
Day +100	PB*	Lineage specific chimerism	Up to 6-8 mL red/green CPT tubes	BPC→DLM
Day +100	PB*	FACS Immune Eval	4 panels (up to 5-8 mL red/green CPT tubes)	BPC
Day +100	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +100	PB	FACS, seq, intracell markers	Viably frozen PBMC: 5 x 10^6 aliquots (up to 5-8 mL CPT)	BPC
Day +100	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +180	PB*	Lineage specific chimerism	Up to 6-8 mL red/green CPT tubes	BPC→DLM
Day +180	PB*	FACS Immune Eval	4 panels (up to 5-8 mL red/green CPT tubes)	BPC
Day +180	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +180	PB	FACS, seq, intracell markers	Viably frozen PBMC: 5 x 10^6 aliquots (up to 5-8 mL CPT)	BPC
Day +180	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +365	BM*	FACS Immune eval	4 panels (1-10 mL green top tube, viably freeze remainder)	BPC
Day +365	PB*	Lineage specific chimerism	Up to 6-8 mL red/green CPT tubes	BPC→DLM
Day +365	BM	FACS, seq, intracell markers	Viably frozen cells: 10 x 10^6 aliquots (1-10 mL green top tube)	BPC
18 months	PB*,%	TBNK	1-3 mL lavender tube	BPC
18 months	PB*	Lineage specific chimerism	Up to 6-8 mL red/green CPT tubes	BPC→DLM
18 months	PB	FACS Immune eval	4 panels (up to 5-8 mL red/green CPT tubes)	BPC

Day/Time point	Spec Type	Correlative Study	Volume[a]	Location of initial processing/specimen analysis
18 months	PB	FACS, seq, intracell markers	Viably frozen PBMC: 5 x 10^6 aliquots (up to 5-8 mL CPT)	BPC
18 months	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +365 and yearly thereafter	PB*	FACS Immune eval	4 panels (up to 5-8 mL red/green CPT tubes)	BPC
Day +365 and yearly thereafter	PB*,%	TBNK	1-3 mL lavender tube	BPC
Day +365 and yearly thereafter	PB	FACS, seq, intracell markers	Viably frozen PBMC: 5 x 10^6 aliquots (up to 5-8 mL CPT)	BPC
Day +365 and yearly thereafter	PB	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Day +365 and yearly thereafter	PB*	Lineage specific chimerism	Up to 6-8 mL red/green CPT tubes	BPC→DLM
Post-HCT^: at diagnosis of and in follow up of IRIS	PB	MAC/CMV-IRIS (Sereti)	Up to 3-10 mL green top tubes	Sereti
Post-HCT^: at diagnosis of and in follow up of IRIS	PB	MAC/CMV-IRIS (Sereti)	6 mL EDTA tube for plasma storage (1 mL aliquots)	Sereti
Event-based	Bodily fluids (urine, stool, CSF, serosal fluids)	FACS Immune eval, seq, intracellular markers	Viably frozen PBMC in aliquots dictated by specimen cellularity	All samples except stool to BPC Stool samples to Chris Kanakry's lab

Note: Samples sent to the BPC may be analyzed by the NCI Laboratory of Pathology for flow cytometric studies.

a. Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.
* Specimen to be processed and assayed in real time. All other specimens will be stored for future use/batched assays.
% To be performed by BPC if not able to be performed by DLM
^ Recipients with mycobacterial infection and/or CMV infection only, as clinically appropriate
& In lieu of this blood draw, extra cells and plasma received in the PDCMF may be shared with the Sereti lab.
Abbreviations: PB, peripheral blood; BM, bone marrow; PBSC, peripheral blood stem cells

Specimens should be sent via escort to the following labs:

Irini Sereti's Lab
Building 10/11B07 Contact: Maura Manion (301-312-2103)

Blood Processing Core
BPC will be notified via email at NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.1.2 Donor Schedule of Biospecimen Collection for Correlative Studies

Table 2

Day/Time point	Spec Type	Correlative Study	Volume[a]	Lab
Pre-HCT donation	PB*%	FACS Immune eval	4 panels (4-8 mL red/green CPT tubes)	BPC
At HCT donation&	BM/PBSC	FACS Immune eval	4 panels (1-10 mL green top tube, viably freeze remainder)	BPC
Pre-HCT donation	PB%	Cytokines/Proteomics	2 mL plasma in 0.5 mL aliquots (from CPT tubes)	BPC
Pre-HCT donation	PB%	Sorting, sequencing, intracell markers	Viably frozen PBMCs: 5 x 106 aliquots (4-8 mL CPT tubes)	BPC
At HCT donation&	BM/PBSC	Sorting, sequencing, intracell markers	Viably frozen cells: 10 x 106 aliquots (1-10 mL green top tube)	BPC
Pre-HCT donation	PB*%	ATG binding	From red/green CPT tubes for FACS	BPC→ Chris Kanakry lab
At HCT donation&	BM/PBSC*	ATG binding	From green top tube for FACS	BPC→ Chris Kanakry lab

Note: Samples sent to the BPC may be analyzed by the NCI Laboratory of Pathology for flow cytometric studies.

Abbreviations: PB, peripheral blood; BM, bone marrow; PBSC, peripheral blood stem cells
a. Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.
% Collect prior to GCSF mobilization
& Biospecimen collection of BM and PBSCs to be done upon arrival of product to DTM and verification that adequate counts were obtained for clinical use.
* Specimen to be processed and assayed in real time. All other specimens will be stored for future use/batched assays.

For pediatric donors, research sample collection is limited to up to a collective total of 10 mL of blood/marrow/peripheral blood stem cells, collected at the time of clinical donation, for research purposes.

Specimens should be sent via escort to the following labs:

Blood Processing Core
Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.2.1 Storage/Tracking in the CIO (formerly ETIB) PDCMF (obsolete as of November 15, 2022)

- Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.
- Normal donor and recipient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be archived by the CIO (formerly ETIB) Preclinical Development and Clinical Monitoring Facility (PDCMF). All data associated with archived clinical research samples is entered into the CIO (formerly ETIB) PDCMF's Microsoft Excel databases on frozen cells and plasma. These databases are stored on the NCI group drive in the CIO (formerly ETIB) 'PRECLINSERVICE' folder. Access to this folder is limited to CIO (formerly ETIB) clinical staff, requiring individual login and password. All staff in the PDCMF laboratory receive updated NIH/CIT training and maintain standards of computer security.
- The data recorded for each sample includes the subject ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, lymph apheresis, mobilized peripheral blood stem cells, marrow, urine, skin or oral biopsy) as well as box and freezer location. Subject demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/CIO (formerly ETIB) clinical records. As of January 2007, all newly received samples receive a unique bar code number, which is included in the sample record in the PDCMF database. Only this bar code is recorded on the sample vial and the vials will not be traceable back to subjects without authorized access to the PDCMF database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.
- Samples are stored in locked freezers. All samples will be labeled solely with a bar code (which includes the date, and serially determined individual sample identifier). The key will be available to a restricted number of CIO (formerly ETIB) investigators and associate investigators on the protocol. Coded samples will be stored frozen at -20°, -80° or liquid nitrogen vapor phase according to the stability requirements under the restricted control of the PDCM Facility of CIO (formerly ETIB).
- These freezers are located onsite at the PDCMF laboratory (12C216) or in CIO (formerly ETIB) common equipment space (CRC/3-3273). Alternatively, at the discretion of the PI/LAI samples may be stored offsite at NCI Frederick Central Repository Services in Frederick, MD.

5.2.2 Storage/Tracking in the Sereti Lab

- Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be

followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

- Normal donor and recipient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be processed by the HIV Pathogenesis Section (HPS). All data associated with clinical research samples is entered into the HPU's Microsoft Excel databases on frozen cells and plasma stored on the NIAID LIR group drive. Access to this folder is limited to HPS staff.
- The data recorded for each sample includes the subject ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, lymphapheresis, mobilized peripheral blood stem cells, marrow, urine, skin or oral biopsy) as well as box and freezer location. Subject demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/CIO (formerly ETIB) clinical records.
- PBMCs are stored in liquid nitrogen and plasma is stored at -80°. Samples are stored in locked freezers. These freezers are located onsite at the HPS laboratory (11B02)

5.2.3 Specimens for Dr. Chris Kanakry's Lab

- If specimens are not first received and processed in the BPC, specimens may be received directly in the Chris Kanakry Lab.
- Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

- Dr. Chris Kanakry will be the only lab personnel with access to identified subject information.
- He will code each specimen sent to the lab before additional members of the lab work with the specimen.
- Normal donor and recipient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch, may be processed, assayed, and analyzed by Dr. Chris Kanakry' s lab. The key to decode the samples will be restricted to Dr. Chris Kanakry. All data associated with clinical research samples is entered, coded, into the lab's Microsoft Excel spreadsheet. These spreadsheets are stored on the NCI group drive in the CIO (formerly ETIB) 'Kanakry Lab' folder. Access to this folder is limited to staff of the Kanakry lab, requiring individual login and password. All staff in the Kanakry laboratory receive updated NIH/CIT training and maintain standards of computer security.
- The data recorded for each sample includes the subject ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g., peripheral blood, lymphapheresis, mobilized peripheral blood stem cells, marrow, urine, skin or oral biopsy). Subject demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/CIO (formerly ETIB) clinical records and

those identified research data are limited to AIs on the protocol with designated privileges.
- Coded, linked samples may be stored, if not processed fresh, in the Kanakry lab, located in the West wing of Building 10, Rm 1-3840.

5.2.4 Specimens for Blood Processing Core (BPC) (as of November 15, 2022)

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.3 PROTOCOL COMPLETION/SAMPLE DESTRUCTION

Once research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and subject consent, or an OHSRP Exemption determination, or determination that the activity is not human subjects research.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject

withdraws consent for their continued use, at which time they will be destroyed. The staff in the labs outlined above will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The PI, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

Subjects' data must be recorded in the progress notes and flow sheets of the patient chart on the CRIS system of the NIH Clinical Center. Recipients' records will be summarized at key time points on day +28, day +42, day +60, day +100, day +180, day +365, day +548, +2 years, and yearly thereafter through +5 years after allo HCT. Recipients' records should also be summarized during clinic visits between days +29 through +99 (frequency in Study Calendar, Section **3.10**). The records should include interpretations of consultant findings as well as any pertinent supplementary information from outside laboratories, clinical care facilities, or radiology centers. Duplicates of key data may be kept in a research folder maintained by the Experimental Transplantation and Immunology Branch. These protocol notes will serve as the primary source material from which data will be collected for research analyses. The protocol notes will be reviewed by the PI and research nurse for accuracy and will include interpretation of the events prior to each note. This will be done to reconcile inconsistencies or uncertainties in the medical record that understandably occur when evaluating new signs/symptoms.

Data will be prospectively collected and entered in real time into the Cancer Central Clinical Data System database. It is expected that clinical data be entered into the database no later than after 10 business days of the occurrence. The research nurse will verify enrollment data entered into the database for each subject within four weeks of signing consent. Donors will only be entered into the database upon signing the research consent and will not be entered if they only sign the screening consent, as we may screen many potential donors for each recipient but we ultimately only select one donor to participate in the research study. Ongoing data entered into the database will be verified by the research nurse monthly, and at the time of continuing review. Documentation of data verification will be tracked in the database. The PI and research nurse will have access to these data via the web.

Recipients' demographics, disease characteristics, treatment and complication history, and outcomes data will be collected for research purposes. Only protocol-specific concurrent medications (only medications in Section **3.1**, study schema, will be recorded in in the database, unless additional medications are deemed necessary to collect for research purposes by the PI. Data on disease re-evaluation following allo HCT will be collected. All clinical data pertaining to a subject's death while on protocol will be collected. Any protocol deviations or unanticipated problems should be directly reported to the PI. The definition of adverse events (AEs) is in Section **7.1**. The recording of AEs is outlined in Section **6.1.1**, and the reporting of AEs is outlined in Section **7.2**.

The exception to data collection is subjects who are taken off protocol therapy to make use of the Miltenyi CliniMACs $CD34^+$ selection device on a compassionate use basis, in which case all data collection ceases as required for the device's compassionate use.

Data will also be sent to the Center for International Bone Marrow Transplant Registry (CIBMTR).

Note: Per NMDP regulations, and to maintain donor confidentiality, unrelated donor source documents will not be sent to the NIH. The NMDP will maintain all required source documents in accordance with NMDP policies and procedures.

6.1.1 Adverse Event Recording

During the time period between the second step of registration (signing onto the treatment study after screening is complete and eligibility is verified) and day -14 of HCT conditioning, no AEs will be recorded or reported, except grade 5 AEs and unanticipated problems.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be recorded from the start of conditioning (HCT day -14) until return to baseline or stabilization of event. All AEs will be recorded through 30 days after completion of protocol-specified therapy (investigational agents have finished) as outlined in Section **3.12.1**, removal from protocol therapy, or until off study, whichever comes first. The last investigational agent/intervention per protocol is when the last drug per the study schema (Section **3.1**) ends. For most recipients, the last protocol-specified drug will be tacrolimus on day +90. Supportive care therapies, such as infectious disease prophylaxis, immunoglobulin replacement, GVHD treatments, etc, are not considered part of the protocol-specified therapy. Once protocol-specified therapy ends and the 30-day post-completion period of AE recording is completed, most recipients will remain "on protocol therapy", meaning that the follow-up, evaluations, and documentation outlined in the study calendar (Section **3.10**) apply. For these subjects, AE recording beyond the 30-day post completion of protocol-specific therapy will only include those AEs necessary to address objectives of the study, at the discretion of the PI. AE recording that is necessary to address objectives of the study will continue to be collected until the subject is taken off study. As an example, a recipient may develop a Streptococcal pneumonia at 2 years post-HCT. At this point, the recipient would be long finished with protocol-specified therapy and would be at the point in follow-up that not all AEs are being recorded. However, if the PI deems the Streptococcal pneumonia event to relate to objectives of the study, such as immune reconstitution, vaccination response, and infection incidence, these data would be recorded as AEs. By contrast, an uncomplicated E coli urinary tract infection, which is more likely a common event not related to the post-HCT status of the recipient, may be deemed by the PI to

not contribute to the objectives of the study and this AE would not be recorded. The decision about whether to record these late AEs, as long as not an unanticipated problem and/or grade 5 in severity, will be determined by the PI. All AEs that are unanticipated problems and/or grade 5 AEs will be recorded for as long as a subject is on study.

For recipients receiving pre-HCT standard of care therapies as a bridge to transplant, these will be standard drugs and regimens with well-defined toxicity profiles. Therefore, adverse events during this standard of care therapy period will not be recorded, except for grade 5 events and unanticipated problems. This also includes laboratory data before HCT day -14.

For donors, AEs will begin being recorded at the time of the first study intervention (research specimen donation). Because donors will need to remain on study as long as the recipient is on study, but their involvement in the study will be limited to discrete periods of time when they donate specimens, donor AEs will only be recorded for the 30 days after each research specimen collection, in addition to unanticipated problems or grade 5 AEs that occur at any time while on study.

A flow diagram of AE recording, as it relates to the subject's status on the study, is shown in **Appendix D**.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the recipient's outcome.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X__ Coded, linked data in an NIH-funded or approved public repository.
_X__ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
_X__ Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through*:*

_X__ An NIH-funded or approved public repository. Insert name: clinicaltrials.gov; dbGaP.
_X__ BTRIS (automatic for activities in the Clinical Center)
_X__ Approved outside collaborators under appropriate individual agreements.

_X__ Publication and/or public presentations.

When will the data be shared?

_X__ Before publication.
_X__ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

All response evaluations will be analyzed by study arm, with no direct comparisons between arms given the non-random nature by which recipients will be assigned to an arm.

6.3.1 Neutrophil Recovery and Duration of Neutropenia

Neutrophil recovery is defined as a post-nadir absolute neutrophil count ≥ 500/mm^3 for three consecutive measurements on different days. The first of the three days will be designated as the day of neutrophil recovery. Duration of neutropenia will be defined as the number of consecutive days post-HCT of absolute neutrophil count < 500/mm^3. If a patient is neutropenic with absolute neutrophil count < 500/mm^3 prior to HCT, then duration of neutropenia will not be defined, and only neutrophil recovery will be captured.

6.3.2 Platelet Recovery

Platelet recovery is defined as a sustained platelet count ≥ 20,000/mm^3 with no platelet transfusions in the preceding seven days. The first of three consecutive measurements on different days will be designated as the day of initial platelet recovery.

6.3.3 Donor Chimerism

Donor chimerism will be assessed in whole blood and/or marrow, as well as sorted cellular fractions in blood. Mixed donor chimerism is defined as ≥ 5% but < 99% donor cells. Full donor chimerism is defined as ≥ 99% donor cells. Decreasing donor chimerism is defined as a decrease in donor cells (or increase in host cells) between two consecutive assessments that results in < 70% donor cells (or > 30% host cells). Stable mixed chimerism is defined as ≥ 70% donor cells on all chimerism assessments from day +28 on. Split donor chimerism is defined as a difference in donor chimerism percentage between cell lineages, using the parameters outlined above.

Prior to allo HCT, a sample of peripheral blood from the recipient and from the donor will be collected for genetic studies to establish a baseline for subsequent chimerism assays.

6.3.4 Graft Failure

Primary graft failure is defined as < 5% donor myeloid chimerism in blood and/or bone marrow on all evaluations up to and including day +60, in the absence of a recurrent marrow malignancy.

Secondary graft failure is defined as initial blood or marrow donor myeloid chimerism ≥ 5%, declining to < 5% on subsequent measurements. If chimerism assays are not performed or are technically not possible in the setting of declining blood counts and the absolute neutrophil count is < 500/mm^3, this will be counted as secondary graft failure.

For the primary endpoint, graft failure will be assessed at day +180 and will include either primary or secondary graft failure, in the absence of a recurrent marrow malignancy. Death will be a competing risk.

6.3.5 GVHD

Acute GVHD: The cumulative incidences of any grade, grade 2-4, and grade 3-4 acute GVHD will be determined. Acute GVHD will be graded according to the Keystone Criteria of the 1994 Consensus Conference on Acute GVHD Grading.(97) Determination of aGVHD diagnosis and grade will ultimately be based on the Keystone clinical criteria, with biopsies suggested but not required to assist in the diagnosis and biopsies not diagnostic, regardless of histopathologic findings, in the absence of appropriate clinical findings. The time to onset of acute grades 2-4 and grades 3-4 GVHD will be recorded, as well as the maximum grade achieved. In calculating the cumulative incidence of aGVHD, competing risks will include graft failure and death.

Chronic GVHD: The cumulative incidence of chronic GVHD will be determined. Chronic GVHD will be scored according to the 2014 NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD.(98) Eight organs will be scored on a 0-3 scale to reflect degree of involvement. Liver function and pulmonary function test results will also be recorded. The global scoring system should be applied only if the diagnosis of cGVHD is confirmed by either 1) the presence of a diagnostic feature or 2) at least one distinctive manifestation of cGVHD with the diagnosis supported by histologic, radiologic, or laboratory evidence of cGVHD. The cGVHD scoring sheet (**Appendix B**) will be used to score recipients at day +60, +100, +180, +365, and yearly thereafter, or more frequently as clinically appropriate. These data will allow calculation of the NIH global severity score of mild, moderate, and severe cGVHD.

Mild cGVHD involves only one or two organ or sites (except the lungs), with a maximum score of 1 in all affected organs. Moderate cGVHD involves either 1) at least one organ or site with a maximum score of 2 in any affected organ or site or 2) three or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 is also considered moderate cGVHD. Severe cGVHD is a score of 3 in any organ or site. A lung score of 2 or greater is also considered severe chronic GVHD. In lung, FEV1 is used instead of clinical score for calculating the global severity. If the abnormality of any organ is attributed to multifactorial causes including cGVHD, the scored organ should be used for calculation of the global severity regardless of the contributing causes.

In estimating the cumulative incidence of cGVHD, death and graft failure will be considered competing risks.

6.3.6 Survival Endpoints

Overall Survival (OS): OS is defined as the time in whole days from HCT to death from any cause, with surviving recipients censored at the time of last contact.

GVHD-free, graft failure-free survival: An aGVHD event will be defined as grade 3-4 acute GVHD not responsive to seven days of high dose steroids (methylprednisolone 1 mg/kg twice daily). GVHD response will be assessed after seven days of high dose steroid therapy and a lack of response will be defined as a $< 50\%$ reduction in skin rash, stool volume or frequency, and/or total bilirubin, progressive signs or symptoms of acute GVHD, and/or failure to maintain a Karnofsky Score $\geq 70\%$ (adults) or Lansky Score $\geq 70\%$ (pediatrics) performance status. A graft

failure event will be defined as either primary or secondary graft failure, in the absence of a recurrent marrow malignancy. A survival event will be death, with surviving recipients censored at the last follow-up where GVHD and graft failure were assessed.

GVHD-free, relapse-free survival (GRFS): GRFS is defined as the time from allo HCT to death from any cause of other event, with surviving recipients censored at the time of last clinical evaluation sufficient to establish the absence of an event. Events include grade 3-4 acute GVHD, chronic GVHD requiring systemic treatment, relapse, or death.

Event-free survival (EFS): EFS is defined as the time from allo HCT to death from any cause or other event, with surviving recipients censored at the time of last clinical evaluation sufficient to establish the absence of an event. Events include: disease relapse (as defined below), graft failure (as defined in Section **6.3.4**), grade 3-4 acute GVHD, cGVHD requiring systemic therapy, receipt of any post-transplant DCI, or death from any cause.

Transplant-related mortality: TRM will be conservatively defined as any death that occurs outside the setting of the post-allo HCT relapse of a pre-transplant malignancy or lymphoproliferative disorder. Relapse of a prior malignancy/LPD will therefore be the only competing risk for TRM. Thus, conditions present prior to allo HCT, such as end-organ dysfunction, uncontrolled infection, or autoimmunity, immune dysregulation, etc which result in death post-allo HCT will be considered TRM, even if not directly attributable to the transplant procedure or its complications. The cumulative incidence of TRM will be estimated at day +100, +180, 1 year, and 2 years after allo HCT.

Treatment-related Mortality (TxRM): TxRM is defined as any death from the start of conditioning (day -14) onward, outside the setting of relapse/progression of a pre-transplant malignancy or lymphoproliferative disorder. TxRM will be evaluated solely for the purposes of the stopping rules.

6.3.7 Reversal of Disease Phenotype

Reversal of disease phenotype will be assessed at one year and will be based upon the lack of prior disease manifestations documented pre-HCT.

Relapse:

Non-malignant TCP/D disorders: The criteria for disease relapse will vary recipient by recipient and will be defined on a case by case basis.

Lymphoma: The status of the recipient's lymphoma/LPD at the time of allo HCT will be evaluated on pre-transplant evaluations with testing and imaging as appropriate for the specific malignancy. Post-HCT assessment of relapse/progression will be as per the study calendar (Section **3.10**), in addition to as clinically appropriate. Prior diagnostic biopsies should also be reviewed, if available, by NIH pathologists to aid in the distinction between a relapse of a prior malignancy and a secondary malignancy after allo HCT. After allo HCT, response will be evaluated using Lugano classification, reproduced from Cheson 2014.(99) For recipients staged with PET-CT, focal uptake in nodal and extranodal sites that is in keeping with lymphoma, according to the distribution and/or CT characteristics, is considered involvement with lymphoma, including spleen, liver, bone, thyroid, etc. For recipients staged with CT, up to 6 of the largest target lesions that are measurable in 2 diameters (longest diameter, LDi, and shortest diameter) should be identified pre-HCT. A measurable node must have a LDi greater than 1.5

cm. A measurable extranodal lesion should have a LDi greater than 1.0 cm. In recipients with pre-HCT bone marrow involvement, bone marrow aspirate and biopsy are necessary to confirm complete response (CR). If a recipient achieves a post-HCT CR, subsequent bone marrow aspirates and biopsies to document ongoing CR are not required but should be performed as clinically indicated. Institution of any therapy to treat persistent/progressive/relapsed lymphoma, including the withdrawal of immunosuppression or DCI, will be considered evidence of relapse/progression regardless of whether the Lugano criteria are met.

Leukemia: For cases of leukemic presentation, the following will be used to assess for relapse:

a. The reappearance of leukemia cells in the peripheral blood of the pre-HCT immunophenotype consistent with the recipient's prior leukemia
b. > 5% disease in the bone marrow, not attributable to another cause, with immunophenotype consistent with the recipient's prior leukemia
c. The development of extramedullary leukemia or leukemia cells in the cerebral spinal fluid
d. The reappearance of cytogenetic abnormalities presents prior to allo HCT

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each subject while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS//DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI Clinical Director Reporting

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will provide continuous, close monitoring, with prompt reporting of serious adverse events to the IRB. On a weekly basis, the PI and the clinical research team will review clinical and laboratory data from patients who are being actively treated on the trial.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical Hypothesis

Primary efficacy endpoints:

The primary objective of this trial is:

- Separately by arm, to estimate the percentage of recipients with >50% donor T cell chimerism *and* graft-failure free survival at day +180 post-HCT

Secondary efficacy endpoints:

The secondary objectives of this trial are:

- By arm, to estimate the cumulative incidences of acute graft-versus-host disease (aGVHD) at 1- year post-HCT, chronic GVHD (cGVHD) at 1 and 2 years post-HCT, primary graft failure at day +60 post-HCT, secondary graft failure at 1- year post-HCT, and transplant-related mortality (TRM) at day +180 and 1- year post-HCT
- By arm, to determine the kinetics and durability of engraftment and lineage-specific donor chimerism, including:
    - The association between early chimerism data (days +21, +28, +35, and +42) and primary or secondary graft failure at day +60 post-HCT

    - The percentage of donor T-, B-, NK-, and myeloid cell populations at days +28, +42, +60, +100, +180, and 1 year after HCT
- By arm, to estimate the probabilities of event-free survival (EFS), GVHD-free graft failure-free survival (GGFS), GVHD-free relapse-free survival (GRFS), and overall survival (OS) at 1, 3, and 5 years post-HCT
- By arm, to estimate the cumulative incidence of Epstein-Barr virus (EBV), cytomegalovirus (CMV), JC virus (JCV), BK virus (BKV), adenovirus, and human herpesvirus 6 (HHV6) detection in blood at day +100 post-HCT

8.2 SAMPLE SIZE DETERMINATION

Recipients will be enrolled onto the trial in two arms, each of which will receive HCT after RIC (Arm 1, for standard-risk recipients) or IOC (Arm 2, for high-risk recipients). Thus, there will be two separate arms for analysis, each evaluated individually. There will be no direct comparisons among the two arms.

Sample size determination for each of these 2 arms will be as follows:

RIC arm (Arm 1):

In Arm 1, recipients will be evaluated according to the probability of having >50% donor T cell chimerism *and* graft-failure free survival at 180 days post-HCT as a binary outcome. In these recipients, having a 180-day event-free fraction below 50% would be considered unacceptable, and it would be desirable if this fraction could be consistent with 75% or higher. With 25 recipients potentially followed for 180 days post-HCT, an exact binomial test with one-sided 0.10 significance level will have 85.1% power to detect the difference between 50% and 75% who are alive, with > 50% donor T cell chimerism *and* free from graft-failure at 180 days after HCT.

As an early stopping rule for Arm 1, if only 0-4 of the first 10 evaluable recipients on Arm 1 are alive, with > 50% donor T cell chimerism *and* free from graft failure at 180 days after HCT, then this will be considered unacceptable since the one-sided upper 90% confidence bound on 4/10 is 64.6%, which is a very conservative approach to early stopping since 0-3 of 10 has an upper 90% CI bound of 55.2%. If this result of 0-4 successes in the first 10 evaluable recipients is obtained, then no further recipients will be enrolled into Arm 1 as soon as this is able to be determined. At this time, the data would be evaluated and discussed with the IRB to determine if there is a common factor related to the platform, donor type, or disease in the recipients with events and if modifications could and should be made to allow enrollment to continue.

IOC arm (Arm 2):

In Arm 2, recipients will be evaluated according to the probability of having > 50% donor T cell chimerism *and* graft-failure free survival at 180 days post-HCT as a binary outcome. In these high-risk recipients with expected high TRM, having a 180-day event-free fraction below 20% would be considered unacceptable, and it would be desirable if this fraction could be consistent with 55% or higher. With 14 recipients on Arm 2 potentially followed for 180 days post-HCT, an exact binomial test with one-sided 0.10 significance level will have 88.1% power to detect the difference between 20% and 55% who are alive, with > 50% donor T cell chimerism, and free from graft-failure at 180 days after HCT.

As an early stopping rule for Arm 2, if 0 of the first 7 recipients in this arm are alive, with > 50% donor T cell chimerism *and* free from graft failure at 180 days after HCT, then this will be considered unacceptable since the one-sided upper 90% confidence bound on 0/7 is 28.0%, which could demonstrate only very slight improvement over the level which is of interest to improve upon and thus would be considered inadequate. If this result is obtained, then no further recipients will be enrolled into Arm 2 as soon as this is able to be determined.

Summary of Accrual:

With these two arms, there are 25+14=39 potential evaluable recipients (recipients) of interest. Since the recipients to be enrolled onto this protocol will undergo screening on this protocol as well, it is expected that about 1/3 of screened recipients will ultimately be treated on this protocol. Thus, to allow for 39 potential recipients, up to 117 recipients are expected to be screened. In addition, a cohort of healthy hematopoietic cell donors will need to be enrolled onto the trial. To yield 39 donors who are able to be used, up to 60 donors will need to enroll onto the trial. Thus, the accrual ceiling for the trial will be set at 117 recipients and 60 donors, or 177 subjects.

8.3 POPULATIONS FOR ANALYSES

Modified intention to treat: all recipients who receive the intended conditioning regimen as well as HCT will be included in the statistical analyses performed.

8.4 STATISTICAL ANALYSES

8.4.1 General Approach

Separately by arm, the fraction of recipients who are alive, with > 50% donor T cell chimerism *and* graft failure-free at 180 days after HCT will be estimated.

8.4.2 Analysis of the Primary Endpoint

The primary endpoint will be analyzed separately in arm as follows:

Separately by arm, the fraction who have >50% donor T cell chimerism *and* graft-failure free survival at 180 days post HCT as a binary outcome will be estimated and reported along with 80% and 95% two-sided confidence intervals.

8.4.3 Analysis of the Secondary Endpoint(s)

The following describes the individual analyses for each of the specified secondary endpoints:

- By arm, to estimate the cumulative incidences of aGVHD at 1- year, chronic GVHD (cGVHD) at 1 and 2 years, primary graft failure at day +60, secondary graft failure at 1- year, lymphoproliferative disease/lymphoma relapse at 1, 3, and 5 years post-HCT, and transplant-related mortality (TRM) at day +180 and 1 year. This will be done using cumulative incidence curves; 95% two-sided confidence intervals will be reported at each of the time points.

- By arm, to determine the kinetics and durability of engraftment and lineage-specific donor chimerism, including:
    - The association between early chimerism data (days +21, +28, +35, and +42) and graft failure at day +60—this will be done comparing the

fractions who achieve chimerism at the stated days between those who have failed by day 60 or have not, using a Fisher's exact test.
- The percentage of donor T-, B-, NK-, and myeloid cell populations at days +28, +60, +100, +180, and 1 year after HCT—this will be a descriptive analysis.

- By arm, to estimate the probabilities of disease-free, progression-free, event-free, and overall survival at 1, 3, and 5 years post-HCT. These probabilities will be estimated by the Kaplan-Meier method.

- By arm, to estimate the cumulative incidence of CMV, BK, adenovirus, and HHV6 detection in blood at day +100 post-HCT, using cumulative incidence curves, along with 95% two-sided confidence intervals.

8.4.4 Safety Analyses

The following stopping rule will be imposed onto each of the two arms, separately applying to each arm, to provide additional safety monitoring for the trial:

Applies arms 1 and 2, separately: With respect to grade 3-4 acute GVHD non-responsive to 7 days of high-dose steroids, if there are 2 recipients anywhere among the first 10 evaluable recipients in an arm who experience this degree of acute GVHD by day +100, then no further recipients will be enrolled into that arm as soon as that is able to be determined.

Applies to arms 1 and 2, separately: With respect to primary or secondary graft failure among recipients of haplo grafts, if 2 of the first 4-6, 3 of the first 7-9, or 4 of the first 10+ recipients of haplo grafts on an arm experience graft failure (primary or secondary), then that arm will be halted to further enrollment of haplo recipients (recipients with matched donors may continue to enroll), and will undergo review and discussion of the outcomes with the IRB to determine if additional changes could be made to try to improve engraftment of haplo recipients. The enrollment of haplo recipients to the arm will be halted as soon as it can be determined that the stopping rule has been met.

These safety analyses apply only to recipients up until the time they receive, if necessary, a second HCT. Recipients who require a second HCT on study and develop GVHD in that context do not contribute to the GVHD stopping rule, as their GVHD would be associated with the second HCT, which is not the protocol-specified HCT. Similarly, this applies to primary or secondary graft failure occurring after protocol-specified HCT but does not apply to graft failure that occurs after second HCT, such as in the case where a recipient had a failed MUD HCT on protocol therapy and then went on to receive a second HCT with a new, haploidentical donor using a standard, non-protocol therapy HCT approach. In both of these instances, the event (GVHD or graft failure) is not a reflection of the protocol therapy and thus should not contribute to protocol stopping rules.

8.4.5 Baseline Descriptive Statistics

Limited demographic and clinical characteristics of all recipients will be reported by arm.

8.4.6 Planned Interim Analyses

As indicated in each arm, an interim evaluation of the fraction of recipients who are able to attain the primary outcome after a specified point will be undertaken in order to assess whether the respective arm will remain open to accrual for those recipients.

8.4.7 Sub-Group Analyses

None are intended.

8.4.8 Tabulation of Individual Participant Data

No individual participant data are intended to be reported.

8.4.9 Exploratory Analyses

The following are the intended exploratory objectives:

- To determine whether HCT reverses the clinical phenotype of T-cell proliferation and/or dysregulation at 1-year post-HCT. Disease-specific studies of immune system phenotype and function will also be evaluated at 1 year for comparison to the pre-transplant state. The relationship between reversal of phenotype and engraftment of donor cell subsets will be evaluated at 1 year
- To determine the activity of e-ATG against T-cell and non-T cell lineages
- To determine the frequency of virus-associated reactivation, viral infection, viral disease, and viral lymphoproliferation after HCT, including serial assessment of EBV, CMV, HHV6, BK virus, adenovirus, and JC virus through 1-year post-HCT
- To evaluate the incidence of other post-HCT infections in the 1-year post transplant, including complications, treatment, duration, and control
- To determine the incidence of invasive fungal infections at day +180 post-HCT
- To evaluate the dynamics of immune reconstitution and changes in cytokine/soluble marker profiles after HCT, including changes related to the development of viral reactivation, severe infection, virus-associated malignancy, and GVHD
- To evaluate the need for and response to subsequent donor cell infusions after HCT
- To evaluate the development of virus-specific immunity after HCT
- To further elucidate the degree of donor chimerism necessary to reverse the disease phenotype in various T-cell-mediated disorders
- To evaluate the impact of HCT on endocrine function at 1-year post-BMT
- To identify and describe the incidence and clinical course of complications, sequelae, and outcomes of HCT in this heterogeneous and unique patient population, on an individual, case series, cohort, or study level, as appropriate and relevant.

Any of these exploratory evaluations which generate quantitative measures will be done using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

9 COLLABORATIVE AGREEMENTS

9.1 MULTI-INSTITUTIONAL GUIDELINES

The study has split IRB review per the Memorandum of Understanding (MOU) associated with the study. The NMDP IRB is responsible for the review of procedures and consent pertaining to unrelated donors at various NMDP centers. The NIH Intramural IRB is responsible for the review of transplant recipients and related donors that are enrolled at the NIH Clinical Center. As such, the NMDP IRB approval documents and consent documents will be provided to the NIH Intramural IRB for the unrelated donors as informational material only, but do not require review and approval.

It may be noted that donors are asked to consider allowing portons of their samples to be used for research; this research is optional. For the purposes of this optional research, NMDP will develop a research-specific consent. At the time of CR, unrelated donors *who do not agree to research* will be included in the total NIH/CCR accrual to the protocol per OPS; unrelated donors *who do agree to research* will be included in "other domestic site" accrual totals. In both cases, as these donors are only identified by gender, demographic information beyond this will not be provided.

9.1.1 IRB Approvals

The PI will provide the NIH Intramural IRB with a copy of the participating institution's approved yearly continuing review. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NIH Intramural IRB.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

Subjects for this study will be males and females of all races and ethnic groups with disorders of TCP/D who are age 4 years or older. Subjects with human immunodeficiency virus will not be candidates for this study due to the high rate of post-transplant complications in this group. Individuals who are pregnant or breastfeeding will not be candidates for this protocol due to the risk to the fetus or newborn. Donors will be males and females of all races and ethnic groups.

10.2 PARTICIPATION OF CHILDREN

Children (transplant recipients) are included in this study, as disorders of TCP/D may manifest at a young age and allo HCT is a potentially curative therapy that can provide a survival advantage to these subjects. Physicians, nurses, and multidisciplinary support teams of the NCI and CC will provide patient care. The staff of the Pediatric Anesthesiology and Critical Care Team (PACCT) has expertise in the management of children with complex oncologic disorders and complications of therapy and has been actively involved in the care of pediatric allo BMT patients on other NCI protocols. Full pediatric support and subspecialty services are available at the NCI CC. However, recipient subjects younger than 4 years of age are excluded given the inability to provide medical intensive care should the patient require it.

Pediatric donors may be enrolled to collect blood and, at the time of clinical donation, a small aliquot of marrow and/or peripheral blood stem cells. Research participation of pediatric donors is limited to the collection of up to a collective total of 10 mL of blood/marrow/peripheral blood stem cells for research purposes.

10.3 Participation of Subjects Unable to Give Consent

Adult recipients unable to give consent are allowed to enroll in the protocol, given prospect of direct benefit. It is also possible that participants enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study, as potential complications of transplant can include significant neurologic events with potential for permanent loss of capacity, such as stroke/intracranial bleed, malignancy progression in the central nervous system, progressive multifocal leukoencephalopathy, meningoencephalitis, etc. In the event this occurs, the participants may remain in the study because the protocol offers the prospect of direct benefit through highly-specialized care post-transplant and management of complications (Section**10.4**) and should therefore exclude participants only when scientifically necessary. Given the nature and severity of potential transplant- or disease-related issues, excluding participants based on the potential to lose capacity could compromise the objectives of the study. All recipient participants ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see section **10.5.1** for consent procedure.

Donor subjects who become incapacitated or cognitively impaired during the course of the study will come off study since there are no direct benefits to the donor subjects.

10.4 Evaluation of Benefits and Risks/Discomforts

10.4.1 Benefits for Recipients

The recipients may obtain direct benefit from allo HCT, as it is a potentially curative therapy for disorders of TCP/D.

10.4.2 Risks for Recipients

The chemotherapy and immunosuppression utilized in this study carry the risk of both acute and long-term toxicity. In addition, immunosuppression is associated with an increased risk of opportunistic infection. GVHD and graft failure are potential complications of allo HCT that remain significant causes of morbidity and mortality. It is anticipated that these risks will be low for recipients on the RIC arm, but is reasonably expected to be high for the recipients on the high-risk IOC arm. Furthermore, the use of a PTCy-based GVHD prophylaxis regimen is expected to result in comparably favorable rates of acute and chronic GVHD. While it is anticipated that the allo HCT platforms used in this study will be associated with relatively low transplant-related morbidity and mortality (TRM), the procedure nonetheless carries risk. The risks associated with the allo HCT procedure will vary by disease, donor type, graft dose, conditioning arm, and comorbidities, among other factors as outlined in the consent. Acknowledging these limitations in predicting risk, the estimated risk of primary graft failure may range between 2-20%. The risk of acute grade 2-4 GVHD is approximately 20-35%, with a risk of acute grade 3-4 GVHD of less than 10%. The risk of chronic GVHD is estimated to be less than 15-30%. The risk of TRM in the first year after allo HCT is estimated to be 10% for the RIC arm but potentially 20-40% for the IOC arm. Therefore, the recipients eligible to participate

in the study must have sufficiently disabling or life-threatening complications of their disease to justify the risks of HCT. During the peri-HCT period, discomforts include nausea, vomiting, mucositis, fever, anorexia, fatigue/malaise, changes in taste sensation, alopecia, diarrhea, rashes/skin irritation, increased burden of daily medications/pills, cytopenias requiring transfusion support, infection, the discomforts of prolonged hospitalization, the discomforts of post-HCT re-vaccination, and potential need for diagnostic procedures (biopsies, bronchoscopies, gastrointestinal scopes, central line placement, etc.) to guide treatment decisions and/or optimize management. Additionally, immune reconstitution post-HCT can lead to the temporary emergence of new or worsening symptoms related to inflammation. Long-term potential risks include organ dysfunction, secondary malignancies, infertility, endocrine dysfunction, growth retardation in children, and psychosocial issues related to having a serious medical illness and undergoing a treatment that carries the risks described above. Finally, HCT is a slow process of post-HCT immune reconstitution, and it may reasonably take years before the new immune system is fully functional without additional supportive care and, by extension, before complications related to relative immunodeficiency, namely infection, are no longer a risk above that of the general population. In particular, this applies to the risk of opportunistic infection and vaccine-preventable infection, but may also apply to more standard infections, such as community acquired pneumonia. See also Section **4.10** regarding the potential increased risk of bleeding in recipients with *MAGT1* mutation.

There are minimal risks associated with electrocardiogram and echocardiogram as these are both relatively safe procedures. While pulmonary function test are relatively safe, some may feel dizzy or faint from the rapid breathing required for the test.

The risks related to blood drawsn for laboratory testing and research sample collection are minimal and include the discomfort and possible pain of phlebotomy, the potential for bruising, and the potential for lightheadedness or fainting surrounding venipuncture, and the potential for mild anemia after blood draws.

Risk of bone marrow biopsy is generally mild pain but rarely bleeding or infection can occur at the biopsy site. To minimize pain, a local anesthesia will be administered prior to the procedure to numb the area.

There are no risks associated with urine and stool sample collection.

10.4.3 Risks for Related Donors

Risks of research specimen donation are minimal and include the discomfort and possible pain of phlebotomy, the potential for bruising, and the potential for lightheadedness or fainting surrounding venipuncture, and the potential for mild anemia after blood draws.

10.4.4 Benefits for Related Donors

There is a potential benefit for donors, as they may derive psychological benefit from participating in a clinical trial designed to improve approaches to transplant and our understanding of immune reconstitution.

10.4.5 Benefits/Risks for Unrelated Donors

Healthy unrelated donors will be enrolled on this study by NMDP. The stem cell collection aspect of this protocol is not investigational. There is a potential benefit for donors, as they may

derive psychological benefit from participating in a clinical trial designed to improve the health of the recipient. Other potential benefits include the diagnosis of previously unknown illnesses at the time of donor screening. Stem cell donation is a safe procedure that is routinely performed in healthy children and adults. Unrelated donors will be closely monitored and undergo procedures to minimize risks by NMDP.

10.5 Consent and Assent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator A signed informed consent document will be obtained prior to entry onto the study.

The initial consent (screening and treatment) process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinical consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant as described below.

Similarly, the procedures involved in this protocol, with their attendant risks and discomforts, including the optional research portion, will be carefully explained to the matched unrelated donors at the respective donor center as required by the NMDP.

If any new information becomes available relating to risks, adverse events, or toxicities, while subjects are participating in this protocol, this information will be provided orally and/or in writing to all enrolled and prospective subject participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signature:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the subject will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

10.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **Error! Reference source not found.**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **Error! Reference source not found.**.

10.5.2 Consent Process for Minors

Consent will be obtained from parent(s)/guardians of minor children as described in Section **Error! Reference source not found.**.

Where deemed appropriate by the clinician and the child's parent(s) or guardian, the child will also be included in all discussions about the trial and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. The assent process will take place in conjunction with consent; therefore, in person and remote assent are permitted under the same circumstances as in person and remote consent. Children ages 12 to 17 years of age, based on their cognitive ability, will sign the appropriate line in the consent document to attest to assent. Children ages 7-11 years old will provide verbal assent. Children under 7 years of age will not be required to provide assent as they typically do not have the cognitive ability to fully understand the nature of research. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide verbal assent. All children will be contacted after they have reached the age of 18 to determine whether they wish to continue on the trial and informed consent will be obtained from them at that time.

10.5.3 Consent for minors when they reach the age of majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

When we have no ongoing interactions with the participants, including if participants are lost to follow up or withdrawn from the study, we request waiver of informed consent to continue to use data and/or specimens obtained from those individuals.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.

(3) The research could not practicably be carried out without the waiver or alteration.
    a. Subjects lost to follow up cannot be located for consent with regard to future sample use. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only plan to request a waiver of reconsent for those subjects who have been lost to follow-up.

10.5.4 Waiver of Consent for Pre-Screening Activities

Prior to the subject signing the consent for this study the pre-screening activities listed in section **2.2.1** may be performed. We request a waiver of consent for the above listed activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

11 REGULATORY AND OPERATIONAL CONSIDERATIONS

11.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to <study participants, investigator, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB and as applicable, Food and Drug Administration (FDA).

11.2 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of nspection by local and regulatory authorities.

11.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

All research activities will be conducted in as private a setting as possible.

The representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB or Institutional policies.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical sites and by the NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NCI CCR.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research

information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

12 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

There will be no IND obtained for the use of any of the commercial agents used in this study.

This study meets the criteria for exemption for an IND as this investigation is not intended to support a new indication for use or any other significant change to the labeling; the drugs are already approved and marketed and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.

12.1 EQUINE ANTI-THYMOCYTE GLOBULIN (E-ATG, ATGAM)

E-ATG is the purified, concentrated, and sterile gamma globulin, primarily monomeric IgG, from hyperimmune serum of horses immunized with human thymus lymphocytes. Please refer to package insert for more details.

12.1.1 Source

e-ATG is commercially available and will be purchased by the NIH CC Pharmacy Department.

12.1.2 Administration Procedures

Subjects will be premedicated 30 minutes prior to infusion start with diphenhydramine 50 mg oral or IV for adults and 1-1.25 mg/kg/dose up to a maximum of 50 mg for pediatric subjects and acetaminophen 650 mg oral or IV for adults and 10-15 mg/kg/dose up to 650 mg for pediatric subjects. Infusions will be given intravenously over no less than 4 hours. Infusion should preferentially be through a central venous catheter through an in-line filter with a pore size of 0.2 to 1 micron. The use of high-flow veins will minimize the occurrence of phlebitis and thrombosis. Do not infuse a dose of e-ATG in less than 4 hours. Infusion times may be extended up to 24 hours to improve tolerance if necessary. Follow nursing care SOP guidelines for monitoring during the infusion. Anaphylaxis kit should be readily available on the unit during administration.

12.2 PREDNISONE

Please refer to package insert for more details.

12.2.1 Source

Commercially available.

12.2.2 Administration procedures

Oral. Subjects unable to tolerate oral medication may be given an intravenous steroid at equivalent dosage.

12.3 PENTOSTATIN (NIPENT®; 2'-DEOXYCOFORMYCIN)

Pentostatin is an inhibitor of the enzyme adenosine deaminase, leading to cytotoxicity through disruption of DNA synthesis. Refer to FDA-approved package insert for complete product information. Please refer to package insert for more details.

12.3.1 Source

Commercially available. The lyophilized powder will be resuspended according to manufacturer instructions, into a solution of 2 mg/ml concentration (10 mg vial).

12.3.2 Administration procedures

Subjects will receive pre-hydration or post-hydration as specified in Section **3.8.4**. Pentostatin is administered IV, usually over 30 to 60 minutes. No extravasation injuries have been reported to be associated with pentostatin in clinical studies. Procedures for the proper handling and disposal of anticancer drugs should be followed. Spills should be treated with a 5% sodium hypochlorite solution prior to disposal.

12.4 BUSULFAN (BUSULFEX®)

Busulfan is a bifunctional alkylating agent approved for use as a conditioning agent prior to allogeneic hematopoietic stem cell transplantation. The IV formulation is commercially available as Busulfex (Otsuka America Pharmaceutical, Inc.). Please refer to package insert for more details.

12.4.1 Source

For patient administration, IV busulfan is purchased by the NIH Clinical Center Pharmacy Department from commercial sources. The drug is supplied as a clear, colorless sterile solution in 10 ml single use vials. Each vial of busulfan contains 60 mg (6 mg/ml) of busulfan.

12.4.2 Administration procedures

Parenteral busulfan must be diluted prior to use with either 0.9% Sodium chloride or 5% Dextrose Injection. The diluent quantity should be 10 times the volume of busulfan so that the final concentration of busulfan is approximately 0.5 mg/ml. busulfan should be administered intravenously via a central venous catheter as a three-hour infusion every 24 hours.

12.5 CYCLOPHOSPHAMIDE (CYTOXAN®)

Cyclophosphamide is an alkylating agent. It is activated by the liver cytochrome P450 system to cytotoxic metabolites, which form cross-links with DNA. It is cell cycle-nonspecific. Please refer to package insert for more details.

12.5.1 Source

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.

12.5.2 Administration procedures

Procedures for the proper handling and disposal of anticancer drugs should be followed. IV cyclophosphamide will be administered over 2 hours when given on days +3 and +4; these administration procedures do not apply to low-dose cyclophosphamide during conditioning if

changed from oral to IV. Slower rates of infusion may decrease the side effects of headache, scalp pain, nasal congestion, and facial swelling. A fluid intake of greater than 2 L/day is recommended during and for 1 to 2 days after cyclophosphamide administration on day +3 and day +4 (see Section **4.13** for prevention of hemorrhagic cystitis).

12.6 MESNA (SODIUM-2-MERCAPTO ETHANE SULPHONATE, MESNEX®)

Mesna (sodium-2-mercaptoethanesulphonate) is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxasophosphorines, including cyclophosphamide. It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxasophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxasophosphorines. Please refer to package insert for more details.

12.6.1 Source

Commercially available

12.6.2 Administration procedures

Mesna will be administered at a dose determined based on the IV cyclophosphamide dose. Each mesna dose will be administered as a continuous infusion.

12.7 TACROLIMUS (FK506, PROGRAF)

12.7.1 Source

Tacrolimus will be obtained by the NIH CC Pharmacy Department from commercial sources and is available in capsules (0.5 mg, 1 mg, and 5 mg), as a parenteral concentrate for injection (5 mg/mL, 1 mL ampules). Please refer to package insert for more details.

12.7.2 Administration Procedures

Tacrolimus may be given intravenously over 24 hours or orally every 12 hours.

12.8 MYCOPHENOLATE MOFETIL (CELLCEPT®, MMF)

Mycophenolate mofetil is an immunosuppressant that is FDA approved for use in combination with cyclosporine and corticosteroids for prophylaxis of organ rejection after kidney, cardiac, or liver transplant. Mycophenolate mofetil is administered as CellCept (Roche labs). Please refer to package insert for more details.

12.8.1 Source

Commercially available. For patient administration, the intravenous form and the oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

12.8.2 Administration procedures

Any oral formulation should be taken on an empty stomach, 1 hour before or at least 2 hours after meals. Oral formulations should not be administered simultaneously with antacids. Avoid inhalation or direct contact with skin or mucous membranes of dry powder contained in capsules or suspension. IV solutions should be administered over at least two hours through either a peripheral or central vein and should not be administered by rapid or bolus injection.

12.9 FILGRASTIM (NEUPOGEN®, G-CSF)

Filgrastim is a human granulocyte colony-stimulating factor which acts on hematopoietic cells by binding to specific cell surface receptors and regulating neutrophil production, progenitor proliferation, and differentiation. The product differs from the natural form due to its absence of N-terminal o-glycosylation. Please refer to package insert for more details.

12.9.1 Source

Commercially available as filgrastim injection in a concentration of 300 μg/ml in 1ml (300μg) and 1.6 ml (480 μg) vials from the Clinical Center Pharmacy Department.

12.9.2 Administration procedures

Filgrastim is administered by subcutaneous injection into the outer upper arm, abdomen, front middle thigh, or upper outer buttocks. Injection site should be rotated. The pre-filled syringes come with 27 gauge, ½ inch needles with an Ultra Safe Needle Guards. The needle cover of the prefilled syringe contains a dry natural rubber that is a derivative of latex. Filgrastim should not be administered earlier than 24 hours after to or in the 24 hours prior to cytotoxic chemotherapy.

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14 APPENDICES

14.1 APPENDIX A - PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	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.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

ECOG Performance Status Scale		Lansky Play Performance Scale (Children)	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Fully active, normal.
		90	Minor restrictions in physically strenuous activity.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Active, but tires more quickly.
		70	Both greater restriction of, and less time spent in, active play.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Up and around, but minimal active play; keeps busy with quieter activities
		50	Gets dressed, but lies around most of day; no active play; able to participate in all quiet play and activities
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Mostly in bed; participates in quiet activities
		30	In bed; needs assistance even for quiet play.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Often sleeping; play entirely limited to very passive activities
		10	No play; does not get out of bed
5	Dead.	0	Unresponsive; dead

14.2 APPENDIX B – GVHD GRADING AND SCORING

Acute GVHD Staging and Grading

Extent of organ involvement			
Stage	**Skin**	**Liver (bilirubin)**	**Gut (stool output per day)**
0	No GVHD rash	<2 mg/dL	<50 mL/day or persistent nausea (child: <10 mL/kg/day)
1	Maculopapular rash <25% BSA	2-3 mg/dL	500-999 mL/day (child: 10-19.9 mL/kg/day) or persistent nausea, vomiting or anorexia, with a positive upper GI biopsy
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL	1000-1500 mL/day (child: 20-30 mL/kg/day)
3	Maculopapular rash >50% BSA	6.1-15 mg/dL	Adult: >1500 mL/day (child: >30 mL/kg/day)
4	Generalised erythema plus bullous formation	>15 mg/dL	Severe abdominal pain with or without ileus
Grade	**Skin**	**Liver (bilirubin)**	**Gut (stool output per day)**
I	Stages 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stages 2-4
IV	Stage 4 or	Stage 4	-

Abbreviations: BSA = body surface area; GI = gastrointestinal; GVHD = graft-versus-host disease.

Chronic GVHD Diagnosis and Staging

Diagnostic signs and symptoms of chronic GVHD are those that establish the diagnosis of chronic GVHD without need for further testing or evidence of other organ involvement. *Distinctive* signs and symptoms of chronic GVHD are those that are not sufficient in isolation to establish a diagnosis of chronic GVHD, where additional testing such as biopsy is needed to establish the diagnosis. *Other features or unclassified manifestations* of chronic GVHD define rare, controversial, or nonspecific features of chronic GVHD that cannot be used to establish the diagnosis. *Common* features are those that are seen in both acute and chronic GVHD. Further details regarding the diagnosis and staging of chronic GVHD are in the National Institutes of Health Consensus Development Project 2014 Working Group Report.

Signs and Symptoms of chronic GVHD

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of chronic GVHD)	Distinctive* (Seen in chronic GVHD, but Insufficient Alone to Establish a Diagnosis)	Other Features or Unclassified Entities†	Common‡ (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 punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	
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			
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 × upper limit of normal ALT > 2 × upper limit of normal
Lung	Bronchiolitis obliterans diagnosed with lung biopsy BOS§	Air trapping and bronchiectasis on chest CT	Cryptogenic organizing pneumonia Restrictive lung disease‖	
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to fasciitis or sclerosis	Myositis or polymyositis¶	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 cardiomyopathy	

ALT indicates alanine aminotransferase; AIHA, autoimmune hemolytic anemia; ITP, idiopathic thrombocytopenic purpura.
* In all cases, infection, drug effect, malignancy, or other causes must be excluded.
† Can be acknowledged as part of the chronic GVHD manifestations if diagnosis is confirmed.
‡ Common refers to shared features by both acute and chronic GVHD.
§ BOS can be diagnostic for lung chronic GVHD only if distinctive sign or symptom present in another organ (see text).
‖ Pulmonary entities under investigation or unclassified.
¶ Diagnosis of chronic GVHD requires biopsy.

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

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

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

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

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

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

Photographic Range of Motion (P-ROM)

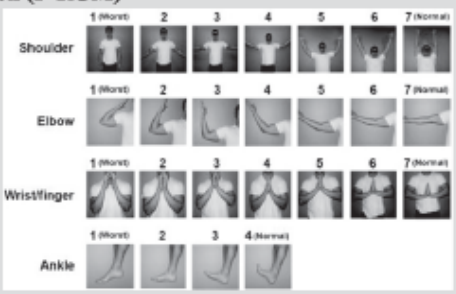


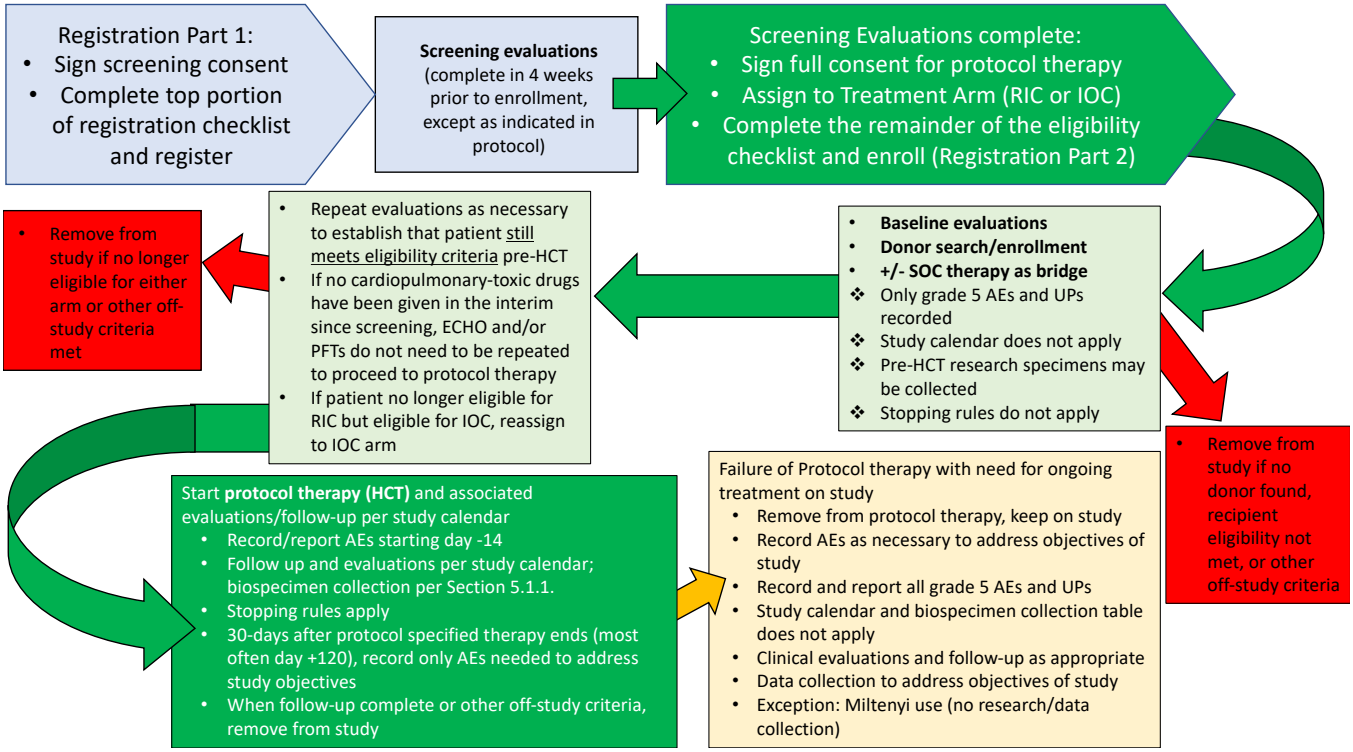
Figure 1. Organ scoring of chronic GVHD. ECOG indicates Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; ULN, normal upper limit. *Weight loss within 3 months. †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. ‡To be completed by specialist or trained medical providers (see Supplemental Figure). **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.

NIH Global Severity of chronic GVHD
Mild chronic GVHD 1 or 2 Organs involved with no more than score 1 *plus* Lung score 0
Moderate chronic GVHD 3 or More organs involved with no more than score 1 OR At least 1 organ (not lung) with a score of 2 OR Lung score 1
Severe chronic GVHD At least 1 organ with a score of 3 OR Lung score of 2 or 3
Key points: 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).

14.3 APPENDIX C – STANDARD CLINICAL ORDER SET

The standard clinical order set will be used for the evaluation of patients on CIO (formerly ETIB) protocols at time points not specified in the protocol. This will include laboratory evaluations that are commonly ordered for CIO (formerly ETIB) subjects (chemistries, drug levels, common hematology labs, cultures, viral PCRs, basic immunology/serology studies, urine studies, stool studies, surveillance cultures, etc.), basic imaging studies such as CT scans, ECHOs, ultrasounds, etc.; routine procedures such as central line removal, NP swabs/washes, or VAD placement, pulmonary function tests, and consults such as dental, dermatology, and gynecology. The order set will be reviewed and approved at least annually by the PI and may be altered, with PI approval, as needs of the branch change.

14.4 APPENDIX D – FLOW DIAGRAM OF SUBJECT STATUS ON STUDY



14.5 APPENDIX E: SCHWARTZ FORMULA

Creatinine clearance calculation (mL/min/1.73m^2) = [length (cm)x k]/serum creatinine

k=0.55 for children ages 4 to 13 years old and adolescent females 13-18 years old

k=0.7 for adolescent males ages 13-18 years old